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THE AMERICAN PHYSIOLOGICAL SOCIETY

Sixty-third Annual Meeting

CLEVELAND, OHIO, APRIL 29–MAY 3, 1951

(An asterisk following an author's name indicates "by invitation")*

Metabolic and pharmacologic studies in glia and nerve cells and components. L. ABOOD,* M. CAVANAUGH,* R. D. TSCHIRGI* AND R. W. GERARD. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Measures of enzyme content and metabolic activity of gross neural regions are of limited value because of the heterogeneous cell population, especially the admixture of glia with neurones. Studies on separated cell species, even on cellular components, are highly desirable. We report results on rat cortex, cord and sciatic and on chick cord tissue cultures. Cord white matter is easily separated by hand from grey, and the white—containing glia but no neurones—homogenized and differentially centrifuged into lipid, soluble, 'mitochondria', and nuclear fractions. Cortex and nerve are directly homogenized and centrifuged. Cultures, averaging 20γ of healthy multiplying microglia, are tested individually; have a Q_{O_2} (per mg. dry weight culture) of about 15 and a $Q^{N_2}_{CO_2}$ of about 50. Glucose and succinate are oxidized, glucose glycolysed. IAA inhibits growth at between 1 and 5γ (per 0.2 cc.), atropine at 10, ACh at 50, carbonyl Ch at 15, adrenaline at 10, lobelin at 10, dilaudid at 30; Na acetate was inactive. Prostigmine and eserine inhibit some at 5 and 30 respectively, less with ACh also present. pH effects were negligible between 5.0 and 8.0. In CNS homogenates, oxidative and phosphatase activity as well as half the content of ribosenucleic acid and of phospholipin, is in the mitochondrial fraction while glycolytic activity is in the soluble fraction. Desoxynucleic acid is in the nuclear fraction. Per unit fat-free dry weight, most enzyme activity is about 50% greater in cortex than in cord white. The same oxidative, glycolytic and Krebs cycle intermediates are present in both grey and white, and in like amounts, except for 2-fold higher inorganic P and triose P in cord. The content will be reported of 16 intermediates, mostly phosphorylated, in whole brain and cord, of N, of lipid P and nucleic P, and of 7 oxidative and glycolytic enzymes, in the fractions of cortex grey and cord white; and the oxygen uptake with 6 substrates and the glycolytic rates of these fractions. Oxidative phosphorylation has been demonstrated for cord, a P/O ratio of 2–2.3 being attained with several substrates, a ratio of 1.3 with succinate.

Ventricular rate as a function of auricular rate: effect of vagal and sympathetic impulses. G. H. ACHESON AND HOWARD LEVITIN.* *Pharmacology Dept., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.*

In dogs weighing 12–20 kg., under Dial-urethane anesthesia, with open chest and artificial respiration, the auricle was driven from electrodes near its tip. Auricular and ventricular rates were counted from auricular electrograms and electrocardiograms over 0.1 minute starting at least 5 seconds after the beginning of a 20-second period of driving the auricle at each frequency studied. The highest auricular rate which the ventricle would follow (X) and the lowest auricular rate at which the ventricular rate was one-half the auricular rate (Y) were determined carefully. The ratio of Y to X usually lies between 1.2 and 1.8 and rarely approaches 2.0. At X the ventricular rate is the highest (270–350 beats/min. in the decentralized heart) encountered at any auricular frequency. This ventricular rate is occasionally reached again at double the auricular rate of X; at higher auricular rates the ventricular rate again falls. Usually, however, the auricle will not follow twice the driving rate of X. In the decentralized heart, maximal stimulation of the vagi decreases, and that of the sympathetic nerves increases, the values of X and Y. At increasing frequencies of vagal stimulation up to 28 per second (beyond which fatigue is evident), X is reduced progressively down to between 54 and 70. The ratio of Y to X remains in the same range. During vagal stimulation X remains the highest ventricular rate observed; with the higher vagal frequencies, the ventricular rate of X may again be reached at 2, 3, and 4 times the auricular rate of X.

Effect of anesthetics on isolated cat auricle. LOUIS J. ACIERNO* AND JOSEPH R. DIPALMA. *Depts. of Medicine and Physiology, State Univ. Med. Center, Brooklyn, N. Y.*

The auricle was suspended in Locke's solution maintained at a temperature of 30°C. The pH was held at 7.5 by bubbling through the solution at a rapid rate a mixture of 95%O_2/5%CO_2. A special

dual pulse square wave stimulator, oscilloscope and optical recording permitted mensuration of excitability, refractory period and contractility. Ether, chloroform and cyclopropane were bubbled separately through the solution at rates ranging from 2 to 100 ml/min. A fresh muscle was used in each determination. Excitability and contractility were depressed by all the anesthetics in proportion to the dose. Ether had the greatest effect and cyclopropane the least. The refractory period was shortened by all three in direct relation to the concentration. The results, especially in view of the shortening of the refractory period, explain, in part, the frequency of arrhythmias following the use of anesthetics. However, the greater incidence of cardiac irregularities after chloroform and cyclopropane as compared to ether is not explained, since, in this study, there appears to be little difference in their actions.

Studies on shock following experimental myocardial infarction in dogs. CLARENCE M. AGRESS,* MARVIN J. ROSENBURG,* MAXWELL J. BINDER,* A. SCHNEIDERMAN* AND WM. G. CLARK. *Cardiovascular Lab., Veterans Administration Center, Los Angeles, Calif.*

A method has been devised to produce shock following myocardial infarction in anesthetized dogs. Catheters are placed in the right auricle and the innominate artery. Recordings are made through Statham Strain gauge manometers by an Offner oscillograph. The heart is exposed and oxygen administered with a Dann resuscitator. Blood volumes are determined with Evans blue dye. A catheter attached to a Statham manometer is inserted through the left carotid artery into the left ventricle; it is withdrawn until the ventricular pressure curve just changes to a central arterial curve. Autopsy has shown the catheter tip to lie immediately distal to the uninjured semilunar valves. The aorta is occluded distally by finger pressure, complete occlusion obliterating the innominate pulse wave. Plastic beads in methyl cellulose gel are injected during aortic occlusion, thus embolizing the coronary circulation. Microscopic examination of organs has shown beads only in the heart. *Conclusions: 1*) Myocardial infraction accompanied by shock persisting for several hours can be consistently produced, thus permitting a long period for study of the coronary shock syndrome. *2*) Aortic occlusion alone, or methyl cellulose into the coronary or systemic circulation, or bead injection into the systemic circulation, will not cause a significant fall in blood pressure. *3*) Bead embolism of the coronary arteries will produce a prompt fall of blood pressure to shock levels, a decrease in the blood volume, electrocardiographic changes consistent with coronary insufficiency, and gross and microscopic evidence of myocardial infarction.

Preference for methionine-supplemented casein (versus unsupplemented casein) by rats on a self-selection regime. ERRETT C. ALBRITTON, LOUIS P. MUNAN* AND HENRY L. MCCORKLE.* *Dept. of Physiology, George Washington Univ. School of Medicine, Washington, D. C.*

The ability of rats to differentiate between casein and a methionine-casein mixture was tested. Male rats, 20 each of the Sprague-Dawley, Long-Evans, and Wistar strains, were grouped separately by strain in 6 large cages and each cage of 10 animals was offered continuously for 19 days two containers of vitamin-free casein, to one of which DL-methionine was added to make a 1% mixture. This constituted the animals' sole source of protein. The intake of supplemented and unsupplemented casein was recorded daily for each cage, and the mean difference calculated and expressed below in terms of grams of protein/100 gm. of rat/day:

STRAIN OF RAT	N	MEAN DIFFERENCE	S. E. OF MEAN DIFFERENCE	*t*	*P*
Sprague-Dawley	38	0.23	.032	7.2	< .01
Long-Evans	38	0.26	.056	4.6	< .01
Wistar	38	0.69	.070	9.9	< .01

All of the strains studied consumed in significantly greater quantities the casein to which methionine had been added. (Supported by a grant from the Natl. Insts. of Health, Public Health Service.)

Blood pooling in acute portal hypertension. ROBERT S. ALEXANDER, W. STERLING EDWARDS* AND JAY L. ANKENEY.* *Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Studies of acute portal hypertension have been carried out on anesthetized and splenectomized dogs while directly recording portal blood flow and the simultaneous splanchnic inflow. It has been found that sudden elevation of portal pressure leads to an immediate cessation of portal flow followed by a resumption of flow which remains significantly below the inflow rate for a considerable period of time. Sudden lowering of portal pressure from hypertensive levels results in a transient gush of excess blood followed by a considerable period in which the outflow remains elevated over the inflow. These results indicate that two types of blood pooling occur: *a*) a small volume which rapidly equilibrates with the portal pressure and which probably represents distension

of the major venous channels and *b*) a much larger volume of blood which is pooled in regions which equilibrate relatively slowly with portal pressure. The anatomical locus of this latter pooling has not been ascertained.

Further studies of thoracic cord lesions on conditioned reflexes in dogs. WILLIAM F. ALLEN. *Dept. of Anatomy, Univ. of Oregon Med. School, Portland.*

In confirmation of last year's report: *1*) Transections of the pyramidal tracts on one side of the cord eliminated the ipsilatoral hindleg conditioned reflex, but allowed a conditioned walking reflex of the hindlegs. *2*) Hemisection of the cord together with contralateral sectioning of the lateral pyramidal tract and a portion of the ventral pyramidal tract, abolished the positive conditioned reflex for either hindleg; but permitted a generalized conditioned reflex, involving head, foreleg and trunk movements. Additional (*1*) and (*2*) lesions resulted in other variations of these generalized responses. Since the first and all successive applications of the negative conditioned stimulus eliminated these complex conditioned reflexes as effectively as the original hindleg conditioned reflex, this suggests usage of a complex inhibitory mechanism. Other dogs in which the reticulospinal fibers were severed in greater numbers ventrally than dorsally, required only a few trials to re-establish the negative conditioned reflex in comparison to the dogs previously reported. This early effect on the negative reflex may be due to increased excitability of the limb, accounted for by the fact that the inhibitory fibers are concentrated ventrally in the cord. Furthermore, complete sectioning of the cord, except for 1 lateral pyramidal tract and 1 or 2 ascending tracts, produced no effect on the positive or negative conditioned reflex for the hindleg on the side of the intact lateral pyramidal tract. It is concluded that pyramidal tract impulses fire the cord nucleus to elicit the hindleg conditioned reflex, but the negative conditioned reflex for the hindleg does not result from extrapyramidal blocking of the activity of this nucleus. This effect takes place at a higher level; presumably, the cerebral motor cortex is the area suppressed.

Oxygen consumption during and after tamponade. NORMAN R. ALPERT (introduced by WILLIAM W. WALCOTT). *Dept. of Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

Under ether anesthesia a polyethylene catheter was inserted in the pericardium and the chest was closed without a pneumothorax. After recovery from the anesthetic the control gaseous exchange was measured with the Scholander respirometer. Oil was injected through the catheter until the mean femoral arterial blood pressure fell to 60–80 mm. Hg where it was maintained for one-half hour. During the succeeding one-half hour the pressure was kept at 50–70 and at 40–60 mm. Hg in the final one-half hour. Throughout this period and for 2 hours after the removal of the tamponade the total gaseous exchange was determined and measurements of pericardial and inferior vena caval pressures were made. In 15 unanesthetized dogs tamponade depressed the oxygen consumption by 20–96% of the control level. In the first 12 minutes of recovery, the oxygen consumption showed a sharp increase, then a decrease, the former sometimes amounting to twice the control value. The decrease was followed by a prolonged rise with a slow return to pre-tamponade values. Recovery was sometimes incomplete after 2 hours. During tamponade the respiratory quotient always rose above that of the control and in the terminal 4 minutes of the stress, values ranging from 1.5–8 were observed. The period of low blood pressure was associated with a 50–300% increase in pulmonary ventilation. This increased ventilation persisted into the early and middle phases of recovery. The excess oxygen of recovery has been compared with the oxygen deficit during tamponade.

Acclimatization of mice to discontinuous exposure to 25,000 feet. PAUL D. ALTLAND AND BENJAMIN HIGHMAN. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

Fifty male Swiss mice were exposed to 25,000 feet simulated altitude 4 hours daily beginning at age 50 days. Exposed mice and unexposed controls were killed at intervals through 1 year; none died due to altitude exposure. The mice appeared unaffected by hypoxia; body weights approximated controls; males were fertile. Hematocrit (tail blood, Van Allen, 2000 rpm, 30 min.) values of controls were 52 to 55 after age 85 days. After 3 to 4 weeks hematocrit of exposed mice increased to approximately 70, declining slightly after 40 weeks. Exposed mice showed no significant gross lesions. Testicular weight was unaffected and no microscopic changes in testis, epididymis or seminal vesicles were found. Slight to moderate hemosiderin deposition occurred in renal convoluted tubules, Kupffer's cells, or pulmonary septal cells in 5 of 7 mice exposed over 300 times, but in none of 6 age controls. In exposed mice all organs showed vascular engorgement. The average diameter of renal glomeruli was increased due to capillary engorgement. This increase corresponded roughly to that calculated necessary for circulatory system as a whole to accommodate the increased blood volume, estimated from hematocrit,

assuming little change in total plasma volume (Merino, *Blood*, 1950). There were no renal infarcts, cardiovalvular and arterial lesions as reported in similarly exposed rats (*Arch. Path.*, 1949). The adult mouse is the first mammal reported able to acclimatize favorably to discontinuous exposures to altitudes above 20,000 feet; it is noteworthy that control mice had relatively high hematocrits at ground level.

Sensory interaction. V. E. AMASSIAN (introduced by T. C. RUCH). *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

The effect of somatosensory conditioning volleys on cortical primary responses from visceral afferent (e.g. splanchnic) or other somatosensory sources was analyzed. Deep sodium pentobarbital (Nembutal) or light Dial anesthesia was used in cats. Selecting cortical recording site, conditioning volleys to nerves bearing particular spatial relationships to test nerve and conditioning-testing shock intervals with precise limits was critical. Results of interaction (inhibition and occlusion) are prominent in microelectrode recordings from subcortical white matter but not from medial lemniscus. Thus, effects of sensory interaction are marked at the thalamic level. Single unit recordings from a thalamocortical axon confirm that a thalamic cell may be prevented from firing by a conditioning volley which does not itself fire the cell (inhibition). Interaction also occurs in the cortex for a single shock to the corresponding region in somatic area I blocks a primary response in area II from peripheral stimulation. At brief intervals the blocking is mainly cortical while at longer intervals a subcortical component is present. If high frequency conditioning stimuli are used (rates at which thalamic transmission just fails), blocking and its components are almost abolished (cf. masking described by Marshall *et al.*) suggesting that conditioning and testing volleys utilize similar pathways to the thalamus but are in separate nerve fibers. The interaction data derive from volleys in A fibers faster than the delta group and traversing the posterior columns. They orgainate in either touch or pacinian corpuscles. Such data may apply to problems of spatial and modality discrimination. (Aided by State of Washington Research Funds for Biology and Medicine.)

Metabolic changes in dogs following transections of central nervous system at various levels. EVELYN ANDERSON, DAVID MCK. RIOCH, KATHRYN KNOWLTON* AND WEBB HAYMAKER.* *Natl. Insts. of Health, Bethesda, Md., Chestnut Lodge Sanitarium, Rockville, Md., and Armed Forces Institute of Pathology, Washington, D. C.*

The role of the central nervous system in pituitary-adrenal function is being investigated. Observations on the metabolism of carbohydrate and protein following transections of the central nervous system at spinal and midbrain levels in the dog will be presented as well as measurements of adrenal-pituitary function. Decerebration at the midbrain level in the dog causes a decrease in carbohydrate tolerance which lasts for two or three weeks. This occurs whether or not the hypothalamus is left intact or is destroyed by hemorrhage following the transection. No disturbances in carbohydrate tolerance are observed following bilateral decortication.

Reticular influences on sensory and thalamic synchronization in olfactory bulb. A. ARDUINI AND G. MORUZZI (introduced by H. W. MAGOUN). *Dept. of Physiology, Univ. of Pisa, Italy.*

High voltage (250 V), 30/sec. waves are recorded from the olfactory bulb of the 'encéphale isolé' cat when air is blown into the ipsilateral nostril. These synchronized sensory responses, previously described by Adrian (1942), are abolished by anemia or by destroying the olfactory bulb. The resting activity is instead characterized by fast waves (about 60/sec.) of lower amplitude. Both sensory responses and resting activity are unmodified when the olfactory bulb is centrally isolated and are therefore independent from the thalamus. Centrally induced synchronization of the olfactory bulb results from 'recruiting' stimulation of thalamic intralaminar areas or may be associated with the spontaneous spindles of the 'cerveau isolé'. The recruiting responses sometimes wax and wane simultaneously in the cerebral cortex and in the olfactory bulb and disappear from this structure as soon as the bulb is centrally isolated. High frequency stimulation of the brain stem activating system or of thalamic recruiting areas suppresses the centrally induced synchronization of the olfactory bulb, possibly through intralaminar mechanisms, leaving the athalamic activity unaffected. Only in few cases, 'spike and wave' patterns (Fortuyn and Jasper, 1947) were recorded during the recruiting response of the olfactory bulb and then the wave component was found to be selectively abolished by appropriate reticular stimulation.

Influence of environmental temperature and thyroid status on reproductive organs of young female mice. FOUAD ATALLA* AND E. P. REINEKE. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Groups of young female mice were housed at temperatures of 24°, 30° or 35°C. for a period of 4 weeks. In addition certain groups at the several environmental temperatures were fed a ration

containing either 0.1% thiouracil or a small percentage of synthetic thyroprotein (.005 to 0.2%). All groups receiving thyroprotein reached maturity earlier than their controls, as indicated by earlier opening of the vagina and onset of estrous cycles. Histological examination of the ovaries revealed numerous corpora lutea and growing follicles in thyroprotein-fed groups, with the greatest number present at the 0.025% level. Control ovaries showed principally follicles, with corpora occurring in only one case. Ovary weights decreases slightly, but not significantly, in the thyroprotein-treated mice while uterus weights showed a significant increase. The progesterone type response observed in uterine sections is correlated with the luteinization observed in the ovary. Elevation of the environmental temperature to 30° or 35°C. did not produce significant changes in weights of the ovaries or uteri of normal mice. Histologically, these organs showed retarded development, however. Thiouracil feeding at all 3 temperatures resulted in ovaries showing only follicular development. Vaginal smears showed many of these animals to be in continuous estrus. The results are believed to indicate that in slightly hyperthyroid mice, gonadotropic hormone from the anterior pituitary is predominantly luteinizing, while in hypothyroidism follicle stimulating hormone predominates.

Cardio-vascular adjustments to pressure changes in left ventricle and peripheral arteries. D. M. AVIADO, JR., T. H. LI, B. CALESNICK AND R. BELL, JR. (introduced by S. GOLDSCHMIDT). *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*

In an attempt to investigate the physiological significance of the Bezold-Jarisch reflexes, the perfusion method which demonstrated pressoreflexes from right auricle, pulmonary arterial bifurcation and pulmonary veins (*Federation Proc.* 9: 255, 1950) is now utilized to study reflexes from the left side of the heart. In dogs (morphine and chloralose anesthesia) with intact circulation, temporary partial ligation of the root of the aorta just above the coronary orifices manifests two phenomena. The first is an abrupt bradycardia that is unaffected by cutting aortic depressor but abolished by vagotomy. Increasing the left ventricular pressure alone in a perfused left side heart isolated from the rest of the circulation maintained by donors and pumps causes a similar reflex bradycardia and reflex vasodilatation of its vascularly isolated perfused leg. The pressoreceptor activity of the left ventricle is qualitatively similar to activities of the carotid sinus and aortic arch pressoreceptors and also to the Bezold-Jarisch reflex from the heart. The other phenomenon is observed following the release of the aortic ligation. The recovery of the aortic pressure distal to the ligature assumes an S-shaped curve—an abrupt rise followed by a fall and a recovery in 1 to 2 minutes. This curve is exaggerated after carotid denervation and vagotomy, and is unaffected by adrenalectomy or by constant cross-cerebral circulation. A similar recovery curve results when perfusion is stopped and then resumed in the isolated vascular system of a leg. It is unaffected by denervation and is unrelated to blood oxygen supply. It appears to be a local adjustment of vessels consisting of a constriction when flow is low and dilatation when high. These adjustments are also seen in splanchnic and cerebral beds.

Alteration of diuretic response to Salyrgan by anoxia and ACTH. D. R. AXELROD (introduced by JOHN MACLEOD). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

The inhalation of a mixture of 8% oxygen, 92% nitrogen reduced the response to mercurial diuretics in the trained, unanesthetized dog. The associated hyperventilation reduced plasma pCO_2 and bicarbonate and increased blood pH. Plasma sodium, potassium and chloride were relatively unchanged. To distinguish between anoxia *per se* and the alterations of pH, bicarbonate and pCO_2 as causative factors in the reduced diuresis, a gas mixture of 6% oxygen, 5% carbon dioxide, 89% nitrogen was given. Although equally severe anoxia developed, blood pH, plasma bicarbonate and pCO_2 remained normal. The response to mercurial diuretics was reduced. Since anoxia is known to stimulate release of ACTH, the relationship between the reduced response to a mercurial diuretic during anoxia and the effect of ACTH was investigated. A dose of ACTH given prior to a mercurial diuretic markedly reduced the diuretic response. These experiments suggest that the reduced mercurial diuresis during anoxia may be due to release of ACTH. (Aided by a grant from the Life Insurance Medical Research Fund.)

Effect of ascorbic acid pretreatment on leukocyte response and survival of adrenalectomized rats exposed to stress. H. BACCHUS, C. A. TOOMPAS AND M. H. HEIFFER (introduced by C. E. LEESE). *Dept. of Physiology, George Washington Univ. School of Medicine, Washington, D. C.*

Adrenalectomized female Wistar rats were used in two experiments. In the first experiment one group received 'pretreatment' with vitamin C (injectable, 150 mg/100 gm.) in 3 intraperitoneal injections; the second group received saline injections. Both groups were later given single subcutaneous injections of epinephrine (0.03 mg/100 gm.). Total and differential leukocyte counts showed that the rise of eosinophils observed in

saline pretreated rats was not observed in the vitamin pretreated animals. This is in marked contrast to the prevention of stress-induced eosinopenia in nonadrenalectomized (intact) rats by ascorbic acid pretreatment (BACCHUS AND TOOMPAS. *Science*, in press). In the second experiment two groups of adrenalectomized rats received pretreatments as those above. Both groups were exposed to cold (−4° to −1° C.) and observed for survival. At the end of 10 hours all of the animals died; organ weights of the groups were identical. Lipids, and glycogen (detected by Bauer-Feulgen and Gage methods), were at a minimum in the livers. Marked pigment deposition, suggestive of erythrocyte destruction, was observed in the spleens and livers of both groups of cold-stressed animals. These findings suggest that the known protective action of ascorbic acid in rats requires the presence of the intact adrenal; and that a close alliance between the ascorbic acid and eosinophils in the blood exists.

Mechanism of antidiuretic action of crystalline ferritin in dogs. S. BAEZ, A. MAZUR AND EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Med. College and New York Hosp., New York City.*

This report is concerned with a further analysis of the antidiuresis produced in dogs by the intravenous administration of crystalline ferritin (90–300 μg. N/kg.). A close correlation exists between the extent of antidiuresis and the blood level of ferritin as quantitated by the rat mesoappendix assay for vasodepressor activity. The association of both the vasodepressor and antidiuretic activities of ferritin with its sulfhydryl form was substantiated by experiments in which inactivation of both of these biological effects was achieved by converting the ferritin -SH to the disulfide form by aerobic incubation with normal liver slices or by specific chemical agents. At the height of ferritin antidiuresis the urine contained antidiuretic activity, demonstrated by injecting intravenously into another dog a concentrate equivalent to a 15-minute urine sample. The antidiuretic activity was inactivated by thioglycollic acid and therefore appeared to be Pitressin-like and, on the basis of other evidence as well, not due to ferritin *per se*. Additional evidence for the posterior pituitary mediation of ferritin antidiuresis is provided by the absence of an antidiuretic effect with ferritin in 7 dogs with diabetes insipidus following stalk resection of the pituitary. Studies are also in progress on the relative antidiuretic effectiveness of ferritin and other biologically active -SH compounds administered both by the intravenous and intracarotid routes.

Effect of cholesterol feeding on dogs with reduced liver function (Eck fistula). ROBERT O. BAILEY (introduced by W. B. BRADLEY). *Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

Adult dogs maintained on a diet of constant composition were used for this study. They were divided into four groups as follows: *Group I*—2 normal dogs given by stomach tube 3 cc. of corn oil/kg. daily. *Group II*—4 normal dogs given 3 cc. of corn oil containing 0.3 gm. cholesterol/kg. daily. *Group III*—2 Eck fistula dogs given 3 cc. of corn oil/kg. daily. *Group IV*—6 Eck fistula dogs given 3 cc. of corn oil containing 0.3 gm. cholesterol/kg. daily. Plasma lipids and liver function studies were carried out every 14 days. At the conclusion of the experiment the composition of the liver lipids was determined on all animals. Lower than normal plasma lipid values were found in the Eck fistula dogs prior to cholesterol feeding. The average plasma cholesterol of *group II* increased from 215–255 mg.% after 165 days of cholesterol feeding while the increase in *group IV* was from 122 mg.% to 400 mg.%. The total cholesterol/lipid phosphorus ratio of plasma remained between 12 and 13 for *groups I* and *III*. This ratio rose to 15 in the dogs of *group II* and averaged 25 for the dogs of *group IV*. Following Eck fistula formation in 2 normal dogs receiving cholesterol in oil, the total plasma cholesterol rose while the phospholipids fell. Cholesterol feeding depressed the rose bengal dye clearance and elevated the serum phosphatase of Eck fistula animals but not of normal dogs. The livers of the cholesterol fed, Eck fistula dogs contained 31% fat and 6.1% cholesterol (wet weight), while those of normal dogs fed cholesterol averaged 8.3% fat and 0.61% cholesterol. The foregoing results are interpreted as indicating that the Eck fistula dog has a decreased ability to catabolize cholesterol and to synthesize phospholipids.

Stimulation and elimination of anterior thalamic nuclei in man and cat. H. BAIRD,* B. GUIDETTI,* V. REYES,* H. T. WYCIS* AND E. A. SPIEGEL. *Depts. of Experimental Neurology and of Pediatrics, Temple Univ. School of Medicine, and St. Christopher's Hospital for Children, Philadelphia, Pa.*

In view of the favorable effect of excision of the cingular gyri in certain psychoses, bilateral lesions of the related anterior thalamic nuclei were produced in psychotic patients. These nuclei were stimulated prior to their destruction by electrolysis. For comparison the same procedures were carried out in cats. Certain similarities could be observed in the results of the procedures on the

cingular gyrus and on the anterior thalamic nucleus. Autonomic effects (particularly alterations in blood pressure and respiratory inhibition) were seen in man on stimulation, and the state of excitation of some psychotic patients was diminished after electrolytic lesions. In cats, stimulation produced similar vegetative effects, and extensive lesions induced a transitory state of catalepsy and a reduced reactivity to noxious stimuli. Further animal experiments are now in progress to determine whether structures adjacent to the anterior thalamic nuclei play a part in the effects observed and to study the possible relation of such effects to afferent or efferent connections between the anterior nuclei and the cingular gyri.

Status of the conversion ratio as a measure of thyroid activity. KENT F. BALLS, JOHN M. PHILLIPS AND JAMES D. HARDY (introduced by WM. M. PARKINS). *Endocrine Clinic, Dept. of Radiology, Hosp. of the Univ. of Pennsylvania, and Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.*

In 1947 Chaikoff, Taurog, and Reinhardt found the rate of conversion of I131 into the protein bound iodine fraction of plasma decreased in thyroidectomized rats but increased in normal animals previously injected with thyrotropic hormone. These authors advanced the rate of conversion of radioactive iodine into protein bound iodine as a measure of thyroid activity. Clark, Moe and Adams found this method useful in human beings. We have performed this measurement in 150 patients having widely different levels of thyroid activity, and have compared these findings with the clinical evaluation, BMR, serum protein bound iodine level, and the uptake of radioiodine by the thyroid gland in these same subjects. In the diagnosis of hyperthyroidism, we have found the conversion ratio to be at least as accurate as the serum protein bound iodine level and more accurate than the uptake of radioiodine by the thyroid gland. In the diagnosis of hypothyroidism we have found the conversion ratio unreliable with the low doses of I131 which we used (200 microcuries). Nevertheless, it is to be remembered that the BMR, serum protein bound iodine level, conversion ratio, and thyroid uptake of I131 measure different facets of thyroid physiology. Thus the several tests frequently complement each other, and under certain circumstances all may be useful.

Thyroxine effects on tissue metabolism. S. B. BARKER AND H. M. KLITGAARD.* *Dept. of Physiology, State Univ. of Iowa, Iowa City.*

Reports in the literature indicate that thyroxine increases the oxygen consumption of most of the tissues of the body. This effect is more pronounced in the thyroidectomized animal than in the normal; the former thus offers a sensitive test object. In order to determine whether any organ, such as liver, is essential to the metabolic response of other tissues to thyroxine, oxygen consumption studies have been conducted on liver, kidney, diaphragm, heart and skeletal muscle excised at varying times after a single thyroxine injection into thyroidectomized rats. The 3 types of muscle showed a response at 12 hours, while liver and kidney had not been affected. At 24 hours all 5 tissues studied exhibited 15 to 65% increases in oxygen consumption, and thereafter roughly followed the elevation for the whole animal. The increase in metabolism of these muscle tissues caused by thyroxine does not appear to require prior activation of either liver or kidney. This evidence may be considered as supporting the concept of some specialized action of thyroxine directly in each tissue, although the hormone may cause the release from liver of enzymically active substances such as proteins or co-factors.

Application of isotopes to study of extra- or intracellular positions of sodium and potassium in tissues of young and adult chicks. J. S. BARLOW* AND J. F. MANERY. *Dept. of Biochemistry, Univ. of Toronto, Toronto, Ontario, Canada.*

There is in the skeletal muscle of 3-day-old chicks a large 'excess' sodium, i.e. sodium in excess of that which exists in the extracellular phase in ultrafiltrate concentrations. Although the small 'excess' sodium found in adult muscle is often considered to be inside the fiber, this view seems untenable when applied to young-muscle for the following reasons: *1*) The amount is large, resulting in a high intracellular total base; *2*) in soaking experiments the entire sodium equilibrates with Na^{24}, K on Li; *3*) the speed of penetration of intravenously injected Na^{24} into the entire Na phase is faster than that of K^{42} into the entire K phase. If the 'excess' sodium does exist in the extracellular phase the nature of the anion binding it is unknown at the present time. In general Na^{24} entered the entire Na-containing phase of other tissues, (heart muscle, gizzard, liver and brain) faster than K^{42} entered the K-containing phase. The rates of penetration of the isotopes into young chick tissues were compared with the entrance rates into tissues of the adult. The existence of an effective blood-brain barrier in a 3-day-old chick was clearly demonstrated.

Cell division block of sea-urchin (Arbacia) eggs and its reversal by ATP. ROBERT C. BARNETT (introduced by FRANKLIN C. MCLEAN). *Marine*

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Since 1895, it has been well known that sea-urchin eggs fail to divide in the absence of oxygen. If energy is necessary to promote cell division, then in an environment where there is no oxygen, perhaps this energy might be artificially provided. It has been found at reduced oxygen tensions such that the divisions of the eggs are reduced to 50% of normal, that the addition of 10^{-5} M adenosine triphosphate (ATP) would restore the division rate to normal. Compounds that lack the 'high-energy' phosphate bond such as inorganic phosphate, adenylic acid, and ATP hydrolyzed in 1 N HCl for 7–9 minutes, failed to reverse the anoxic division block. Compounds, other than ATP, possessing the 'high-energy' bond such as pyrophosphate, and inorganic triphosphate were without effect at the concentrations used. It thus appears that ATP may play an important role in division in these marine eggs.

Glucose metabolism by the red cell. GRANT R. BARTLETT (introduced by E. M. MACKAY). *Scripps Metabolic Clinic, La Jolla, Calif.*

A tracer quantity of uniformly C^{14}-labeled glucose was added to defibrinated human blood and the mixture incubated at 38°C. for 3 hours or until approximately half of the glucose had been utilized. Lactic acid accounted for 80–90% of the glucose used. Ten to 20% of the radioactivity of the metabolized sugar remained in the red cells after exhaustive washing with isotonic saline to remove all of the glucose and lactate. The activity was extracted with trichloroacetic acid and precipitated from the neutralized extract with barium ion. A water-insoluble barium salt, separated from ATP and inorganic phosphate, carried 80–90% of the non-diffusible radioactivity of the red cells. Although not yet conclusively established, the compound appears to be a phosphoglyceric acid. The effects of various inhibitors on the accumulation of this carbohydrate intermediate will be described. No trace of radioactivity from glucose appeared in polysaccharide, protein or fat, a fact of some interest in regard to ideas on fermentative assimilations. Rat blood gave essentially identical results.

Adrenal cortical activity and hematological changes in man during cold acclimatization. DAVID E. BASS, DAVID C. FAINER, RICHARD K. BLAISDELL AND FARRINGTON DANIELS, JR. (introduced by A. H. HEGNAUER). *QM Climatic Research Lab., Lawrence, Mass., and Army Med. Dept. Research and Graduate School, Washington, D. C.*

Mechanisms of cold acclimatization possibly referable to altered adrenal cortical activity were investigated in 12 men exposed to 60°F. for 12 days, and after a 10-day interval, for another 5 days. (Men were nude 7¼ hours daily.) Adrenal cortical activity was assessed by direct eosinophil counts and uric acid-creatinine ratios. Also measured: erythrocyte counts, hemoglobin, hematocrit and erythrocyte water. During cold exposures, blood was drawn on *days 1, 4, 8* and *11*, immediately before entering the cold (8 A.M.), and after 4 hours exposure. Controls were at 85°F. Eight A.M. eosinophils fell significantly below controls on the 4th and 8th days, respectively; the 11th day of initial exposure and the 4th day of re-exposure did not differ from controls. Acute exposure (4 hours) resulted in no marked eosinophil responses. Seven to eight A.M. uric acid-creatinine ratios were unchanged throughout. However, urine voided from 8 A.M. to noon had higher U-A/Cr ratios during cold exposures than during corresponding control periods. Erythrocytes gained water after 4 hours on the 4th day of initial exposure and the 1st day of re-exposure periods only. Morning hematocrits were higher during the exposure phase than controls. Hemoconcentration during 4 hours exposure was significantly greater on the 1st than on the 11th day. Data suggest modifications of degree of hemoconcentration with extended cold exposure. Evidence of altered adrenal cortical activity is equivocal.

Determination of arterial oxygen tension in man by equilibration through intact skin. J. PERCY BAUMBERGER AND ROGER B. GOODFRIEND.* *Physiology Dept., Stanford Univ. Med. School, Stanford, Calif.*

The finger is immersed in phosphate buffer solution at 45°C. in a closely fitting test tube. The high temperature keeps the finger in hyperemia so that the capillary blood is virtually arterial. Given sufficient time, the pO_2 of the buffer solution will become equal to the pO_2 of the arterial blood. The pO_2 of the buffer is determined at appropriate intervals by means of the polarographic method. Oxygen can pass through the skin into the capillaries in either direction judging by the fact that the buffer will increase in pO_2, when in contact with the skin, if it has a low initial pO_2 and conversely will diminish in pO_2 when initially this is high. The equilibrium value is found to agree with the pO_2 of alveolar air. Variations of the alveolar air were obtained by breathing O_2-N_2 mixtures or by rebreathing; the pO_2 by the finger method agreed with alveolar air. The O_2 transferred across the skin is about 2×10^{-3} cu.mm/min/mm.Hg pressure gradient/cm.^{2} skin surface and it therefore requires 15–60 minutes immersion of the finger for equilibrium to be established. The procedures involved are not disturbing to the subject and this suggests usefulness in certain

respiratory and circulatory conditions. The development of a similar method for pCO_2 is underway.

Adrenal alteration induced by oxygen at high pressure. JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan, Ann Arbor.*

Repeated short exposure of baby albino rats to oxygen at high pressure (OHP) interferes with normal growth (BEAN AND BAUER. Unpublished data). This, and the recognition that conditions of stress alter the adrenal cortex suggest that such exposure to OHP might also induce gross and microscopic changes in the adrenals. Experiments were therefore carried out in which each of several litters of baby and young adult rats were divided into test and control groups. The test animals were exposed to oxygen at about 70 pounds for several minutes followed by a long rest interval, four times per day over periods of from 2 weeks to 3 months, but in order to avoid convulsions and possible death this schedule could not be rigidly adhered to. The adrenals of test animals exposed over the longer periods followed by 7 months of nonexposure, were markedly larger and heavier than their controls—some by as much as 90%—and grossly were the color of liver. Glands from animals killed immediately following the termination of the shorter exposure schedules were also distinctly larger and heavier than their controls but the color was only very slightly darker than normal. The results indicate that the adrenals are grossly altered by such exposure to OHP, that these changes induced by prolonged exposure are apparently irreversible, and that the occurrence of oxygen convulsions and permanent motor disabilities induced by OHP may be causally related to these glandular changes.

Influence of alterations in pulmonary arterial CO_2 on pulmonary volume and blood flow. JOHN W. BEAN AND W. PORTER MAYO.* *Dept. of Physiology, Univ. of Michigan, Ann Arbor.*

These experiments were performed on isolated perfused dog lungs set up in a constant temperature bath. Transfer of the lung to the perfusion circuit entailed only a momentary interruption of its circulation. Blood of the desired P_{CO_2} was administered from reservoir bottles of heparinized blood previously equilibrated to the desired tension and maintained at constant temperature and pressure: Extreme sensitivity of pulmonary flow to very minute changes in perfusion pressure necessitated rigorous control of the latter. Blood flow was recorded kymographically by thermopile and volumetric methods along with perfusion and intrathoracic pressures. Lung ventilation was achieved by negative pressure pump to 'intrathoracic' space and was recorded by spirometer through tracheal connection with soda-lime cartridge. A shift from normal (3% CO_2 in O_2) to high CO_2 (8%–15% in O_2) caused *1*) a predominant decrease in outflow of blood frequently preceded by a slight initial increase; *2*) an increased volume of the quiescent lung (plethysmographically recorded); *3*) a decreased intrapulmonary air volume (spirometer record in ventilated lung); *4*) a decreased blood inflow similar to but not identical with that of the outflow. All effects were reversible. The decreased flow is attributed to vasoconstrictive action of CO_2 and possibly also to contraction of parenchymal muscle; the increased lung volume and decreased intra-pulmonary air volume is ascribed to an increased blood reservoir capacity of the lung with a consequently decreased alveolar volume and to a contraction of parenchymal muscle.

Estimation of cardiac output by dye method with an ear oximeter. EARL F. BEARD,* JOHN W. NICHOLSON, III* AND EARL H. WOOD. *Mayo Clinic and Mayo Fndn., Univ. of Minnesota, Rochester.*

The differential changes in the absorption of light in the red and near infra-red produced by addition of T1824 to blood, recorded by whole blood cuvette and earpiece oximeters (*Science*. December 15, 1950), have been used to obtain continuous estimations of concentration of the dye in blood flowing from the radial artery and in blood of the heat-flushed ear. To assure complete and constant arterial oxygen saturation, subjects breathed 99.6% oxygen; 30–50 mg. of dye was injected intravenously, and quantitative absorption curves were recorded. The contours of simultaneous cuvette and earpiece curves from 17 normal subjects and 59 patients with cardiovascular abnormalities were qualitatively similar, suggesting the possibility of quantitation of ear-oximeter curves. For each of 16 normal subjects, an earpiece calibration curve was constructed assuming linearity and that equal concentrations exist in the ear and a peripheral vessel 5 minutes after injection. Direct determinations of dye concentration in radial artery blood withdrawn 5 minutes after injection were used for calibration in 10 'basal' subjects, and cardiac indexes obtained by replotting earpiece time-concentration curves semilogarithmically averaged 3.1 ± 0.2 while those from simultaneous cuvette-oximeter curves averaged 3.2 ± 0.2 l/min/m.^{2} When direct determinations in oxygenated venous blood were used for calibration in the other 6 subjects (heart rate 67–96/minute) the cardiac index averaged 4.7 ± 0.5.

Cation exchange resin therapy in edema: its limitations in advanced renal disease. WILLIAM S. BECK AND HOWARD C. GOODMAN (introduced by W. A. SELLE). *Med. Service, Wadsworth Hosp.,*

Veterans Administration Center, Los Angeles, Calif.

Because compensated acidosis is said to develop during cation exchange resin therapy used to reduce sodium intake, we have investigated possible hazards of its use in patients with edema associated with advanced renal disease where, presumably, renal mechanisms which compensate for acidosis are impaired. WIN 3000, an ammonium-liberating cation exchange resin, was administered in gradually increasing doses to 2 patients with intractable edema, one with chronic congestive failure due to mitral stenosis and one with chronic constrictive pericarditis. A diuresis was obtained in both. The CO_2 combining power remained normal and there occurred no significant changes in serum levels of Na, K, Cl, Ca, P, Mg and urea. During treatment, excretion of fixed base decreased while ammonia excretion increased. Weight loss exceeded that previously achieved with dietary sodium restriction and intensive mercurial therapy. Resin was similarly administered to 2 patients with chronic glomerular nephritis in degenerative or 'nephrotic' stage with intractable edema and serum creatinine levels over 5 mg.%. Both developed acidosis with elevated serum P and urea and decreased Ca. Diuresis occurred in only one; however, his profound acidosis (CO_2 4.4 mM/l.) necessitated alkali administration. The second developed moderate acidosis (CO_2 13 mM/l.). In contrast to the patients with normal renal function, no rise in urinary ammonia excretion above the low control level occurred. The results indicate the value of an ammonium liberating resin in intractable edema, but emphasize its dangers in advanced renal disease.

Rapid CO_2 determination with the Pauling O_2 analyzer. VIVIAN G. BEHRMANN AND FRANK W. HARTMAN.* *Labs., Henry Ford Hosp., Detroit, Mich.*

In routine expired air analysis, a gradual apparent increase in O_2 values, resulting from CO_2 loss through rubber bags, was observed. Transfer to synthetic butyl bags, which are practically impervious to CO_2 allowed consistent O_2 values. This experience suggested the idea that CO_2 calculation might be possible if the apparent O_2 increase on complete CO_2 removal were significant. Since this proved to be true, the feasibility and accuracy of this method has been investigated. The efficiency of ascarite, KOH solution and soda lime in absorption bulbs of variable size and shape was tested. The optimum gas flow rate (200 cc/minute) and time (2–2½ minutes) required for steady readings were determined. O_2 content of the original sample (A) was read after passing the gas through anhydrous $CaSO_4$ (drierite) into the analyzer. O_2 percentage, after CO_2 removal (B) was read by diverting the gas through soda lime, and drierite, into the analysis chamber. CO_2 calculation was made by the relationship: $CO_2\% = (B\text{-}A)/B$. Results on both the C (110–160 mm. range) and the E-2 (0–25% range) models were checked by duplicate Haldane analyses. In 575 analyses (C-Model) by one observer, 73% of the total agreed with Haldane averages within ±.10% while 95% agreed within ±.15%. On the E-2 instrument, 70% of the analyses checked within ±.10% while 90% agreed within ±.15%. A Pauling instrument with a narrower range and/or greater sensitivity should improve the accuracy of these data thus affording complete respiratory gas analysis within 5 minutes.

Fatty acid oxidation in the liver cyclophorase system. HELMUT BEINERT,* VERNON CHELDELIN AND MARIA FULD.* *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

Details are provided of a rapid method for preparing essentially homogenous suspensions of rabbit liver mitochondria, relatively stable, and with high cyclophorase activity (Q_{O_2} = 40 for citrate). Even-numbered fatty acids (except butyric acid) give rise to approximately one mole of acetoacetic acid per mole of fatty acid. Odd-numbered fatty acids below C_7 and isocaproic acid form no acetoacetic acid. These relations were found to hold also for the cyclophorase systems of pigeon and rat livers. C_{14} label from carboxyl-labeled heptylic acid appears in acetoacetic acid added as carrier confirming Weinhouse, Gurin and Geyer. The isotope data may be explained in terms of equilibration of C_2 units rather than of net synthesis of acetoacetic acid. Preparations which oxidize consistently the lower fatty acids ($<C_{12}$) do not oxidize consistently the higher fatty acids. The problems attending the oxidation of C_{16} and C_{18} fatty acids will be discussed. Under appropriate conditions, the methyl and ethyl esters of fatty acids can be oxidized. Phenyl valeric acid can be oxidized to hippuric acid in presence of glycine.

Induction of rancidification in liver tissue by organic mercury compounds. REINHOLD BENESCH AND RUTH E. BENESCH (introduced by HENRY B. BULL). *Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

When one part of normal rat liver was ground with 10 parts of a 0.005 M solution of an organic mercury compound such as *p*-chloromercuribenzoic acid or Salyrgan, a typically rancid odor developed. This process was accelerated by illumination with visible light. Most experiments were therefore performed after illumination of the samples for varying lengths of time, usually 1–3 hours. Fat deterioration was confirmed by the conventional Kreis test, which became strongly positive after one hour of illumination at room

temperature, while control samples remained entirely negative. In addition, formation of peroxides was demonstrated by: *1*) The coupled oxidation of carotene, since added carotene was destroyed much more rapidly in the mercury treated homogenates than in the controls. *2*) The oxidation of Fe^{++} to Fe^{+++} and of HI to I_2 by the extracted liver lipids. *3*) The polarographic reduction of the extracted peroxides. After one hour's illumination the peroxide values of the liver lipids from the treated samples ranged from 15–20, whereas those from the control samples were zero. Perfusion of the intact liver, prior to homogenization with the mercurials, greatly reduced rancidification. Addition of blood partly restored the effect. Addition of ascorbic acid (10–50 mg/100 gm. liver) profoundly accelerated the mercury induced rancidification but had no effect on control homogenates. The amounts of organic mercury compounds used were sufficient to block all the liver -SH groups, as confirmed by amperometric silver titration of the treated samples. However, the induction of rancidification cannot be ascribed to the removal of -SH groups alone, since organic mercaptides such as Thiomerin produced the effect, whereas other -SH reagents, such as thiolsulfinates, alkylating agents and the disulfide Intramine-disuccinate had little or no action.

Influence of diabetes mellitus on formed elements of the blood. GLORIA A. BERARDI* AND ALBERT S. GORDON. *Dept. of Biology, Washington Square College, New York Univ., New York City.*

The effects of pancreatectomy (technique of SHAPIRO AND PINCUS, '36) and of alloxan were studied upon the peripheral blood elements of the rat. Alloxan was administered as a single dose of 175–200 mg/kg. body weight. *Chronic experiments:* In both groups, no significant alterations in red cell counts or fragilities, hemoglobin concentrations, reticulocyte percentages, total or differential white cell counts were observed when determinations were made at 1-week intervals for a period of 6 weeks following initiation of treatment. Counts in animals in which the treatments had induced marked continuous hyperglycemia were similar to those in which the procedures had failed to produce a diabetic state. The injection of 1 dose of alloxan into partially depancreatized rats neither intensified the diabetic symptoms nor produced any significant changes in the peripheral blood counts. *Acute experiments:* A drop in red cell count and hemoglobin accompanied by increased red cell fragility and reticulocytosis occurred as early as 2½ hours following administration of a diabetogenic dose of alloxan. The anemia became marked at 48–72 hours. That alloxan was probably inducing an alarm reaction was seen from the finding that severe lymphopenia and neutrophilia were present at 2½ hours and became maximal at 7 hours following drug administration. At 48 hours, the differential white cell counts had returned to normal. It is concluded that experimentally-induced diabetes mellitus exerts no significant influence upon the peripheral blood picture of the rat. The marked effects induced by alloxan in the acute experiment represent probably a manifestation of its toxicity.

Histochemical detection of fatal anticholinesterase poisoning. A. D. BERGNER* AND S. H. DURLACHER. *Med. Div., Army Chemical Center, and Office of Chief Med. Examiner of Maryland, Baltimore.*

Cholinesterase is stable in post-mortem material and is demonstrable by the Koelle-Friedenwald technique. In mice kept at room temperature after death no change in enzyme activity was noted for 48 hours. Following death from ANTU, 1080, morphine, KCN, CO, arsenic, Nembutal, ether, BAL and acetylcholine, deposition of CuS, indicative of cholinesterase activity, at the site of the motor endplate was not distinguishable from that of the controls. Following death from the anticholinesterases DFP, prostigmine, physostigmine, parathion, TEPP and OMPA, deposition of CuS was partially or completely inhibited. After physostigmine, parathion, TEPP and OMPA less CuS was deposited following an immediate autopsy than when the dead animal was kept for 24 hours at room temperature before autopsy. OMPA was more effective as an anticholinesterase when the animal did not die too quickly. Cholinesterase in human intercostal muscle is demonstrable by this technique. It persisted in post-mortem material for at least 26 hours at room temperature and longer under refrigeration. *In vitro* inhibition of human cholinesterase with DFP is demonstrable also.

Problems concerning functions of frontal agranular cortex. A. J. BERMAN, FRANKLIN ROBINSON AND KARL H. PRIBRAM (introduced by JOHN F. FULTON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

Certain aspects concerning ablations of frontal agranular cortex, namely, importance of locus of lesion with special reference to 'suppressor' areas and mass (or bilaterality) of lesion, remain obscure. Eleven rhesus monkeys were therefore trained in box problems requiring sequential removal of peg and lid to obtain reward. Time scores, alternately using suitable restraints to one arm, were recorded. Complete resection of precentral motor cortex (4 animals) produced severe deficits in fine movements of the contralateral upper extremity, manifested by increased time scores. These deficits receded but persisted for one year

and were increased further by adding homologous lesions in homolateral cortex. Resections restricted to the anterior half of this region (2 animals) produced deficits lasting only 2 months, more obvious in proximal limb muscles. Resections restricted to the immediate precentral region (3 animals) resulted in temporary deficits characterized by incoordination of fine hand movements, interfering briefly with rapid hasp-box performance. Resection of all frontal agranular cortex failed to increase the deficit in problem-box performance. However, bilateral resections of this extent proved to be totally paralyzing. These observations suggest *1*) locus of lesion in precentral motor cortex determines locus of paresis but neither involvement of this cortex nor of other frontal agranular cortex relates to ability to perform these learned tasks, with the exception that more anteriorly placed lesions interfere with serially sequential performance; *2*) mass (and bilaterality) are significant only after a certain threshold has been passed, and then only in affecting duration of disability, awkwardness of performance or totality of paralysis.

Effect of reduced cardiac output on denervated kidney. ROBERT M. BERNE (introduced by CARL J. WIGGERS). *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio.*

Acute reduction of cardiac output was accomplished in closed-chested dogs, anesthetized with pentobarbital or chloralose in from 6–25 days after denervation of the left kidney. In some experiments cardiac output was reduced by a screw clamp and in others by distension of a balloon introduced into the pulmonary artery via a jugular vein. Cardiac output was determined by the Fick principle. PAH and creatinine were used to measure renal plasma flow (RPF) and glomerular filtration rate (GFR), respectively. Sodium was determined by a flame photometer (internal standard). The right kidney served as the control. Renal blood flow (RBF), GFR, sodium excretion and urine flow were nearly equal in the two kidneys after uni-lateral denervation. The pulmonary artery was narrowed to a point giving reduced cardiac output associated with a rise in central venous pressure and only slight fall in mean arterial pressure. RBF fell proportionately less than the cardiac output. The blood flow ratio of denervated to control kidney remained relatively constant with only small flucuations in either direction in some of the experiments. GFR fell to a lesser degree than RBF and returned to or toward control values upon release of the pulmonary stenosis. The change in GFR was approximately equal in both kidneys. Sodium excretion paralleled changes in GFR and urine flow.

Frequency selection in the automatic electroencephalographic control of anesthesia (servoanesthesia). REGINALD G. BICKFORD. *Lab. of Electro-encephalography, Mayo Fndn., Rochester, Minn.*

Previous reports from this laboratory have demonstrated the possibility of regulating the dose of an anesthetic agent by an automatic electromechanical device which uses the electroencephalogram as a measure of depth of anesthesia. The continuous quantitation of the electroencephalogram required for this method has been obtained by electrical integration of the amplified scalp voltage within the frequency pass of the electro-encephalogram amplifiers. Increasing experience with both animal and human anesthesia has revealed some defects in this servosystem. These arise mainly from the fact that the relatively wide band pass of the amplifiers employed has rendered them subject to electrical disturbances other than those produced by the brain. This interference usually occurs at low frequencies (0.5–5 cycles) and at 60 cycles. To improve the reliability of this system, the changes in frequency of the cat's electro-encephalogram with progressive deepening of barbiturate anesthesia were investigated. They were quantitated by the Walter analyzer. These results showed that the maximal change in the energy output occurred at 8 cycles. A frequency-discriminating circuit sharply tuned at this frequency was therefore incorporated in the servosystem described previously (BICKFORD, R. G. *EEG Clin. Neurophysiol.* 2: 93, February 1950). The frequency selective system has been tested on cats (12) and monkeys (3). Barbiturate anesthesia has been maintained in these animals from periods up to 3 days. The new system has the following advantages: *1*) freedom from extraneous interference, *2*) more accurate control of anesthesia, and *3*) greater simplicity in amplifier design. The method has also been applied successfully to the automatic control of barbiturate anesthesia in the human being.

Effect of neurotripsy upon strength of partially denervated muscles. S. P. W. BLACK AND F. E. NULSEN (introduced by J. R. BROBECK). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The effect of tibial nerve crushing upon the strength of partially denervated gastrocnemius and soleus muscles was studied in 9 immature rhesus monkeys (*Macaca mulatta*). A complete section of the peroneal nerve was done bilaterally in order to destroy the opposing action of the muscles causing dorsiflexion of the foot. The power of the gastrocnemius and soleus muscles was then determined by electrically stimulating the sciatic nerve percutaneously and recording the tension devel-

oped by the resultant plantar flexion of the foot. Then a variable, nearly identical weakness in the two legs was produced by bilateral section of one or more lumbar anterior roots and usually the first sacral anterior root also. The consequent decline in strength of the muscles was determined. At an interval varying from 30–79 days after rhizotomy, 7 animals were subjected to a unilateral neurotripsy of the tibial nerve or its branches. Determinations of the strength were then made periodically over a minimum time of 80 days to a maximum of 655 days. The power of the gastrocnemius and soleus muscles on the side of the neurotripsy occasionally, in time, returned in amount greater than that displayed before the crush. The strength of the muscles on the uncrushed side, however, increased even more during that time, presumably by virtue of hypertrophy of those muscle fibers remaining innervated after the rhizotomy. It is concluded that the occasional improvement in strength following neurotripsy did not measure up to the improvement occurring spontaneously in the identically paralyzed control leg.

Acquired resistance to cold exposure in the rabbit and the rat. J. R. BLAIR, J. M. DIMITROFF AND J. E. HINGELEY (introduced by A. D. KELLER). *Physiology Branch, Army Med. Service Field Research Lab., Fort Knox, Ky.*

Groups of 6 rabbits and 12 rats were conditioned to moderate cold exposure for a 7-week period. The rabbits were housed for 20 hours of each day in an ambient temperature of −20°F. (−29°C.), while the rats were housed 16 hours daily at 20°F. (−7°C.). The foregoing ambient temperatures have proved to be the lower range of cold exposure which each of these species, when not previously conditioned to cold, can tolerate without adverse physiological reactions. At the end of the 7-week conditioning period to moderate cold, these groups of animals, along with similar control groups, were exposed to a more severe degree of cold. The rabbits were exposed for an 8-hour period to an ambient temperature of −50°F. (−45°C.), and the rats for 5 hours at 5°F. (−15°C.). The cold-conditioned animals, without exception, tolerated the period of exposure to severe cold without adverse physiologic responses, namely hypothermia and cold injury (frostbite). On the other hand, all unconditioned animals suffered progressive hypothermia and second- or third-degree frostbite. These observations are interpreted as demonstrating *1*) that rabbits and rats, under appropriate conditioning to moderate cold, acquire an unusual resistance to severe cold exposure, and *2*) that these two species have different quantitative physiological tolerances to cold exposure.

Effect of adrenaline on renal excretion of water and sodium. WILLIAM D. BLAKE. *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

Experiments were conducted on unanesthetized dogs in the post-absorptive state and the usual clearance techniques used to estimate renal function. So-called hydropenic dogs were those which received nothing by mouth and an intravenous infusion of 0.9% saline at a constant rate of 0.45 cc/minute. At other times these dogs received initial water and salt loads by stomach tube and an intravenous infusion of 0.5% saline at a rate of 1.5 or 2.7 cc/minute. In the hydropenic dogs 0.004 mg/minute of adrenaline had no effect on urine flow or glomerular filtration rate but caused a marked reduction in the concentration of sodium in the urine with a consequent fall in sodium output. Similar results were obtained when these dogs had received saline by mouth and were infused with 0.5% saline. A third dog (made hypervolemic by 0.9% saline by stomach tube and by infusion at a rate of 2.7 cc/minute) responded to 0.004 mg/minute of adrenaline with an initial fall in filtration rate and urine flow as well as in sodium excretion. During the second 10-minute period of a 20-minute adrenaline infusion filtration rate usually returned to control values and urine flow rose but urine sodium concentration fell and sodium excretion continued to decrease. Adrenaline appears to have influenced tubular activity toward sodium, probably indirectly.

Antispasmodic action of basic esters and quaternary derivatives of β-hydroxy acids. E. W. BLANCHARD, M. H. EHRENBERG,* J. A. RAMP* AND G. R. TREVES.* *Research Lab., Schieffelin and Co., New York City.*

A new series of compounds, basic esters and quaternary derivatives of β-hydroxy acids, have been prepared which possess strong antispasmodic action against acetylcholine and barium induced spasms. The pharmacological activity of 34 of these compounds have been compared with that of known antispasmodics, such as atropine, papaverine, Banthine and Trasentine. Spasms were produced in the isolated rabbit ileum by acetylcholine and barium chloride and in the guinea pig ileum by histamine. None of the compounds showed significant antihistaminic activity. In general the quaternary methiodides showed greater anticholinergic activity than the corresponding tertiary hydrochlorides, but the greater musculotropic action was exhibited by the latter. Several of the compounds possess anticholinergic activity comparable to that of atropine. Others exhibit musculotropic action of the same order as papaverine. Several compounds combine relatively high levels of anticholinergic and musculotropic activ-

ity. Acute toxicities were determined by intravenous and intraperitoneal injections in mice. In general, the quaternary methiodides were about 4 times more toxic than the corresponding tertiary homologs. The intravenous LD_{50} for the tertiary hydrochlorides was in the range of 50–80 mg/kg. whereas the comparable LD_{50} for the quaternaries was 11–25 mg/kg. Complete mydriasis, which wore off rapidly, was shown by several compounds following instillation into the rabbit eye. Preliminary clinical testing of these indicated desirable mydriatic and cycloplegic properties—namely, rapid onset of action and rapid recovery of accommodation. The effects of these compounds on uterine activity, salivary secretion, and on the cardiovascular system were also studied.

Effect of stimulation of renal nerves on renal circulation and function. MELVIN A. BLOCK,* KHALIL G. WAKIM AND FRANK C. MANN. *Div. of Exptl. Medicine, Mayo Fndn. and Mayo Clinic, Rochester, Minn.*

Shielded electrodes were placed around the renal nerves to study the effects of continuous and interrupted electrical stimulation on the circulation and functions of the kidney. Gross observations and India ink injections of kidneys in rats, rabbits and dogs gave evidence of vasoconstriction sufficient to cause the cessation of the flow of blood through the kidney for a few minutes followed by rapid recovery. During this recovery period the ink perfused segments of the kidney, but evidence of a vascular shunt could not be demonstrated. Even though nerve stimulation caused marked blanching of the renal cortex, the medulla remained pink as seen in cross section, but in our experiments a perfusion of only the medulla was not obtainable. During the recovery period, the renal venous blood became cyanotic. Renal arteriovenous oxygen differences determined only in the dog were found to be increased. The extraction ratio of para-amino hippurate (PAH) decreased immediately following stimulation to values ranging from 0 to 66% of the control but returned to control values after 45 to 60 minutes of continuous stimulation. Creatinine and PAH clearance studies during continuous bilateral renal nerve stimulation indicated that the glomerular filtration rate and effective flow of renal plasma decreased greatly during the first half hour but returned to control values after approximately 2 hours. It was impossible to maintain low values for these clearances by interrupted stimulation. The reduction in volume of urine during renal nerve stimulation lasted longer than the decrease in clearances in spite of repeated administration of fluids.

Changes in concentration of phosphorus P^{32} in the organs of the rat following unilateral adrenalectomy. JOSEPH B. BOATMAN, JOSEPH H. SUNDER AND CAMPBELL MOSES. *Addison H. Gibson Lab. of Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Adult female albino rats were unilaterally adrenalectomized, and sacrificed with control animals, 28 days later. Both groups were given 100 μc. P^{32} 24 hours before removal of adrenal in experimental group, and 28 days later, 24 hours before sacrifice of both groups. The first removed adrenal was compared with the remaining adrenal after 28 days and with the adrenals of normal rats for rats retention of P^{32} by dip-counting radioassay. The pituitary, thyroid, thymus, liver, ovary and brain of both groups were weighed and assayed for P^{32} in both groups. Retention of P^{32} in a tissue was compared in terms of concentration ratios (organ's % injected activity/organ's % body weight). Significant increases of the concentration ratios after 24 hours, of P^{32} administered as inorganic PO_4 were seen in the pituitary (+44.5%), thymus (+44.3%), and the thyroid (+40.4%), of the operated group, compared to control animals. No significant changes were seen in the liver, ovary and brain. The remaining adrenal of the operated animals had hypertrophied to 218% of the weight of the removed adrenal, while its concentration ratio was 30.8% less, and 28.3% below those of the control group. It was concluded that adrenal insufficiency created by unilateral adrenalectomy resulted in increased inorganic phosphate utilization by the pituitary, thymus and thyroid and decreased utilization in the hypertrophied adrenal tissue. (This work was aided in part by a grant from the Sarah Mellon Scaife Foundation.)

Production of experimental endocarditis in dogs. J. R. R. BOBB, J. D. M. WARGO,* J. M. SHAFFER* AND M. B. VISSCHER. *Depts. of Physiology and Medicine, Med. School, Univ. of Minnesota, Minneapolis.*

In the exploration of the physiologic changes occurring with heart failure in dogs having large arteriovenous fistulae, many of the animals died with endocarditis (P. S. E. B. M., 75: 9, 1950). As a result, an experiment was started to determine if endocarditis could be produced at will since its production in animals has hitherto been a vexing problem in experimental medicine. Large arteriovenous fistulae (iliac with a femoral or bilateral iliac) were produced in a series of dogs. In 2 weeks, 0.5, 0.05 and 0.005 cc. of a 24-hour broth culture of bacteria were injected daily for 7 days. Six of 7 injected with beta streptococcus developed an endocarditis which was proven at autopsy. The single failure received minimal numbers of bacteria and at autopsy gave clear evidence of having fistulae smaller than originally

intended. Three of 7 dogs receiving alpha streptococcus had endocarditis at autopsy. One of 2 dogs receiving non-coagulating staphloccus had endocarditis. Of 11 normal controls injected for periods up to 43 days with 0.5 cc. of a 24-hr. culture of alpha or beta streptococcus, only one (beta) showed endocarditis at autopsy. Seven fistula control dogs were prepared. No injections were made in these animals. They were kept entirely free of body vermin and reasonably free of intestinal worms. Although 4 were allowed to survive over 5 months, none of these showed endocarditis at autopsy. (This work was done in partial fulfillment of requirements for a Ph.D. degree.)

Coronary artery ligations in anemic and normal dogs. J. R. R. Bobb, J. D. M. Wargo* and H. N. Wright. *Depts. of Physiology and Pharmacology, Med. School, Univ. of Minnesota, Minneapolis.*

In view of the studies of Prinzmetal, the question arose whether the resulting infarct or clinical course after a coronary artery interruption would be influenced if the oxygen-carrying capacity of the circulating blood were markedly decreased. Seven selected pairs of dogs were bled; the heparinized blood centrifuged; one of the pair was given the plasma fraction; the other was given the cellular fraction. This was repeated until the one dog had a hemoglobin below 9 gm/100 cc. of blood. It was not possible to get the plethoric dog's hemoglobin above 15.5 gm/100 cc. At this point the left anterior descending coronary artery was ligated aseptically in the region where it usually gives off the first branch to the anterior left ventricular wall. During the procedure, a procaine solution was given slowly intravenously. In this series, no dogs were lost on the operating table on account of ventricular fibrillation. Three of 4 dogs with hemoglobin levels below 8.3 gm/100 cc. died within 12 hours of operation. Of the 7 dogs with hemoglobin levels above 14.0 gm/100 cc., only one dog died within 12 hours. The remaining 10 dogs were killed. Of these dogs no differences could be seen in the 2 groups in regard to size, distribution or severity of infarction.

Site of action of ethyl carbamate and sodium azide in the living embryonic cell. Joseph Hall Bodine. *Dept. of Zoology, State Univ. of Iowa, Iowa City.*

By means of fractional centrifugation techniques, the effects of ethyl carbamate and sodium azide on the oxygen uptake of the intracellular constituents can be compared with those for the intact grasshopper embryo. Ethyl carbamate and sodium azide produce a marked inhibitory effect upon the oxygen uptake of the intact embryo, its homogenate, the isolated intact nuclei, mitochondria and microsomes. Methylene blue antagonizes the inhibitory action of ethyl carbamate and sodium azide. The 2-4 dinitrophenol, on the other hand, produces a stimulating action only on the intact embryo and no indications of an antagonistic action are evident. Methylene blue exerts its stimulating action at the nuclear membrane while 2-4 dinitrophenol requires the cell to be intact to produce its stimulating effects.

Observations on the flight motor of diptera. Edward Boettiger and Edwin Furshpan.* *Dept. of Zoology, Univ. of Connecticut, Storrs.*

The 'click' phenomenon (*Biol. Bull.* 99: 346) has been produced by electrical stimulation and, mechanically, by properly directed pressure at the wing articulation. Movements of the wings in the click are due principally to the action of a simple lever system consisting of the scutellum and the scutellar bridge. The second axillary sclerite connects the first axillary sclerite to the mesopleural process in such a way that when the process is drawn or pushed in the articulation has stability in only the up or down position. Records of scutellar movements after removal of the wings showed the operation of the click mechanism undamped by wing load. The scutellum moves with increasing velocity in either direction until brought to an abrupt stop. Velocity builds up slowly at first but reaches the click point in one msec. This pattern of movement is believed to be the result of the increasing mechanical advantage at the wing articulation inherent in the click mechanism rather than to the time course of a muscle twitch. Electrical stimulation frequently initiates flight movements. Records of scutellar movement during stimulation show that each stimulus starts a burst of cycles which are damped out unless a new stimulus arrives in time. This effect is due to increasing tension in the direct, nonfibrillar muscles. Increasing frequency produces rough movements which cease with the wings in the clicked down position.

Hypercholesterolemia as a possible adrenal response to ascorbic acid. Walter M. Booker, Frances Mae Dent* and Raymond L. Hayes.* *Depts. of Pharmacology and Oral Medicine, Howard Univ. Colleges of Medicine and Dentistry, Washington, D. C.*

Experiments (in press) have been reported from these laboratories describing increase in serum cholesterol in dogs following ascorbic acid administration. When adreno-cortical extract was administered along with ascorbic acid, greater and more prolonged increases in serum cholesterol were seen. In order to test further the validity of these observations and to propose their significance, it was thought to be important to extend

these experiments to another species (rats) using groups of animals according to the following plan: *1*) Four groups of rats, containing 3 animals in each group were arranged in metabolism cages. All groups, except one (control) received ascorbic acid 10–100 mg. intramuscularly. One hour, 2 hours and 3 hours following ascorbic acid administration groups of animals were sacrificed, bled (blood pooled) and adrenals removed. Ascorbic acid and cholesterol were determined on blood and adrenals. Ascorbic acid was determined also on urine. *2*) Adrenalectomized rats were arranged in groups and treated with ascorbic acid as above. Animals were sacrificed, bled and determinations made according to the above schedule on blood and on urine. *3*) Adrenalectomized animals were arranged in groups and treated with ascorbic acid as above. Each group was given 2.5 dog units (0.05 cc.) adrenocortical extract/100 gm. of body weight. Sacrifices and determinations on blood and urine were made as above. The results showed that in animals whose adrenals were intact, ascorbic acid causes an increase in serum cholesterol, particularly significant in the second and third hour groups. Adrenals from such groups showed steady decline in cholesterol with concomitant increase in ascorbic acid.

In vitro incorporation of labeled leucine into the proteins of rabbit reticulocytes. HENRY BORSOOK, CLARA L. DEASY, ARIE J. HAAGEN-SMIT, GEOFFREY KEIGHLEY AND PETER H. LOWY. *Kerckhoff Labs. of Biology, California Inst. of Technology, Pasadena.*

Rabbit reticulocytes incorporate labeled leucine *in vitro* very rapidly; rates of 10 μEq/gram of protein/hour have been observed. No incorporation occurs in mature erythrocytes or in hemolyzed reticulocytes. The leucine is incorporated into the hemoglobin and into the other proteins of the reticulocyte to about the same degree. In the hemoglobin not more than one % of the incorporated leucine was in end positions with its amino group free. The rate of incorporation is several times faster in serum than in Ringer's solution; the sera of other animals are as effective in this respect as rabbit serum. The rate varies sensitively with dilution of the serum with Ringer's solution. On dialysis of the serum, a small fraction of the activating principle is in the dialysate; the major portion remains with the proteins. The incorporation is nearly completely inhibited by arsenite and dinitrophenol; partially inhibited by anaerobiosis, α,α'-di-pyridyl and diethyldithiocarbamate and slightly, if at all, by arsenate, azide, and hydroxylamine; the above inhibitors were 0.001 M.

Frequency-intensity curves of human muscle. A new method for quantitative electrodiagnosis. H. D. BOUMAN, B. S. BAUMGARTNER* AND K. J. SHAFFER.* *Univ. of Wisconsin Med. School, Madison.*

Frequency Intensity curves for sinusoidal current for both motor and sensory response were obtained by cutaneous stimulation in man, in the range of 1–20000 cycles. Above 1000 cycles the sensory threshold rises much steeper than the motor threshhold. From 1000–20000 cycles much stronger, less painful contractions can be obtained than with commonly employed lower frequencies. Below 1000 cycles curves differ from animal results. The data were plotted according to Hill (n^2 versus I^2 for high frequencies, $1/n^2$ versus I^2 for low frequencies). Straight lines were found. The high frequency line resembles the curve of denuded animal muscle, the low frequency line points downward toward the low frequencies instead of upward. Skin impedance was changed by change of electrode size and by mecholyl iontransfer to the skin. No change in the shape of the curve was found. Direct measurement of skin impedance and phase angle showed that skin impedance cannot explain the difference between human and animal results. The curve from 1000 to 20000 cycles can be used as a quantitative measure of excitability, its slope being proportional to the product of rheobase and chronaxie. In a series of human muscles in different stages of denervation it was found that the slope of the curve increases with decreasing muscle strength. Measurement of the slope of this straight line gives a quantitative determination of muscle excitability in man, free from errors present in other methods in clinical use.

Mechanism of seizures induced by di-isopropyl fluorophosphate (DFP). WILLIAM F. BOUZARTH* AND HAROLD E. HIMWICH. *Medical Div., Army Chemical Center, Md.*

Curarized artificially respired rabbits were prepared for electrical recording from the motor and limbic cortices, thalamus and caudate nucleus. The anticholinesterase drug, di-isopropyl fluorophosphate (DFP), was injected either by vein or in the carotid artery until convulsive patterns were observed in the electroencephalogram. DFP destroys ChE and presumably causes a pile-up of acetylcholine. In the majority of instances grand mal-like convulsive patterns appeared in the following order: first thalamus, then limbic cortex or caudate nucleus and finally the motor cortex. Grand mal-like seizures in the motor cortex occurred when the other three areas exhibited the fit simultaneously, suggesting the necessity for a functional unity within the brain for seizure waves, in the motor cortex. Petit mal patterns were most common in subcortical structures.

Regulation of rate of shortening of myosin B threads by magnesium and calcium. WILLIAM J. BOWEN. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

Skeletal muscle can be dissolved in alkaline KCl solution (Weber's solution). Such a preparation is called natural actomyosin (myosin B). For these experiments it was purified by precipitation in 22 volumes of water, and then redissolved in 0.6 M KCl. Threads prepared by extruding it from a fine orifice retain the ability of the muscle to shorten if treated with ATP. The rate of shortening of short sections of such threads can be followed by means of a microscope equipped with a camera lucida. The rate of shortening is correlated directly with the concentration of ATP. Concentrations of ATP between 0.4 and 0.8 μM cause less than 10% shortening in 5 minutes while concentrations between 1.6 and 3.2 μM cause 30, or more, % shortening. The former range of concentrations of ATP can be made more effective than the latter by adding $MgCl_2$ to the suspending medium in final concentration of 0.03 M. Conversely, $CaCl_2$ causes a drastic decrease in the effectiveness of ATP. If these *in vitro* phenomena prevail in the intact muscle the release of magnesium and calcium from an inactive form could regulate muscular contraction.

Chemical and physical mechanisms involved in muscular contraction. EMIL BOZLER. *Dept. of Physiology, Ohio State Univ., Columbus.*

In muscles previously stored in 50% glycerol, ATP in moderate concentrations causes contraction (Szent-Györgyi), but in high concentrations (0.5%) it produces, under certain conditions, rapid relaxation and a manifold increase in distensibility. Washing the relaxed preparation with saline causes the tension to rise again. Thus both phases of the response of muscle can be produced by ATP. Smooth muscle from the frog's stomach and frog skeletal muscle in iodoacetic acid rigor give similar, but weaker, responses. It may be concluded that the relaxed state depends on the presence of large amounts of ATP, while contraction is due to the disappearance of part of the ATP. The equivalent of normal contraction in the simplified system, therefore, is not the contraction produced by low concentrations of ATP, which starts out with a state of rigor, but rather the contraction of a preparation which has been brought into a relaxed state by ATP and which is washed with saline. The results agree with the assumption that relaxation is due to the resynthesis of ATP and its adsorption by the contractile proteins. That relaxation of skeletal muscle is not a purely mechanical process is shown by the study of the effect of stretching during relaxation (*J. Cell. & Comp. Physiol.* 8, 1936). In smooth muscle, on the contrary, relaxation is controlled by the viscous properties of the contractile elements as shown by the observation that the drop of tension after passive stretch and during isometric relaxation follows the same time course (*J. Cell. & Comp. Physiol.* 18, 1941).

Collective end-plate potentials as analogy of generation of neurocellular current. CHARLES R. BRASSFIELD AND WILLIAM W. STEINBERGER.* *Physiology Lab., Univ. of Michigan, Ann Arbor.*

The isolated sartorius nerve muscle preparation of the frog was used to study the summed activity of motor end plates. The electrical responses were recorded by a d. c. amplifier—cathode-ray oscillograph—camera combination. End plate potentials were produced in the curarized (1:100,000) preparation using both single volleys and repetitive stimulation. Prostigmine produced changes in the end plate potential in response to single volleys in keeping with the theory of the generation of neurocellular current by the neuron cell body. Both the rising and decaying phases of the end plate potentials were prolonged by all concentrations studied (up to 3.3×10^{-5} M). The magnitude of the response was increased as well. With repetitive stimulation, temporal summation of potentials occurred, similar to that described by other workers for sympathetic ganglia. There was a prolongation of both rising and decaying phases as with single volleys. In contrast to prostigmine, acetylcholine and carbon dioxide were found to have a depressing action on the magnitude of the end plate potentials produced by single volleys and by repetitive stimulation.

Lipids in human cervical mucus. MARY ALICE B. BRECKENRIDGE* AND W. T. POMMERENKE. *Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

To further characterize the chemical nature of human cervical mucus and its function in the fertilization process, the lipids of the mucus have been determined throughout the intermenstrual cycle in normal adult women. Total lipid averages 2.9% of the dry weight of the mucus, 0.082% of the wet weight. The average cholesterol content is 0.47% of the dry weight, 0.013% of the wet weight. The lipid phosphorus averages 29 mg.% on a dry weight basis, 0.79 mg.% wet weight basis. Histological staining of mucus smears reveals no localization of these lipids. During the ovulatory phase of the cycle when the total output of the mucus is increased tenfold, the concentration of lipid phosphorus is lowest both on a dry weight and a wet weight basis.

Mechanism connecting impulse conduction and oxygen metabolism in peripheral nerve. D. W. BRONK AND F. BRINK. *Jenkins Labs. of Bio-*

physics, Johns Hopkins Univ., Baltimore, Md.

By the use of inhibitors of oxidative enzymes there have been revealed some details of the reaction mechanism that intervenes between the electro-chemical processes immediately associated with conduction of impulses and the reaction involving oxygen directly. The steady-state rates of oxygen uptake by frog nerves treated with inhibitors were measured at several frequencies of impulses. In addition, the transients between such steady states of oxygen metabolism were recorded. The increment in rate of oxygen uptake at 50 impulses per second was reduced about 50% when nerves were exposed to modified Ringer's solution containing chloretone at a concentration of 2 mM. The percent inhibition was less the lower the frequency of the impulses. The time constant for the rise to steady-state rate at each frequency was prolonged as was that for the return to the resting rate when the imposed activity was terminated. At 2 mM concentration of chloretone the rate of oxygen metabolism of resting nerve was reduced about 15%. At a concentration of 4 mM the resting rate was reduced 40% and there was no increase of this rate during 30 minutes of activity at 100 impulses per second. These results indicate that chloretone inhibits an enzyme in a reaction located between the oxidative reaction directly affected by impulses and the reaction using oxygen. The actions of chloretone on nerve will be contrasted with those previously reported for sodium azide.

Characteristics of muscle responses to pyramidal stimulation. JOHN M. BROOKHART. *Dept. of Physiology, Univ. of Oregon Med. School, Portland.*

The pyramidal tracts, at the level of the medulla oblongata, have been selectively stimulated in decerebrate and lightly anesthetized cats and lightly anesthetized macaques. Multiple shocks, of controllable form, frequency and train duration were applied to the ventral surface of one pyramid or by means of stereotactically oriented electrodes. Contralateral muscle responses were recorded from selective bipolar electrodes imbedded in the reacting muscles and registered on a dual cathode ray oscilloscope. No limb muscles have responded to single shocks applied to the pyramid. The duration of pyramidal tetanization required for threshold activation of a muscle is constant under controlled experimental conditions for any particular animal. This duration threshold varies for different muscles at any one combination of stimulus parameters, but varies with changing stimulus parameters. Preliminary data indicate that duration threshold is characteristic of an entire muscle and does not vary from motor unit to motor unit. Preliminary data may be interpreted as indicating differences in the spinal distribution of large as compared to small diameter fibers of the pyramidal system. (Supported, in part, by a research grant from the National Institutes of Health, Public Health Service.)

Cardiac arrest in dogs following a rapid fall in alveolar carbon dioxide tension. E. B. BROWN, JR. AND FLETCHER A. MILLER.* *Depts. of Physiology and Surgery, Univ. of Minnesota, Minneapolis.*

Mongrel dogs were anesthetized with sodium pentothal and tracheotomy was performed. Control electrocardiograms were made using the three limb leads and the dog was then connected in an open system to a balanced spirometer filled with 30% carbon dioxide in oxygen. A continuous electrocardiogram was made for the first 5 minutes after starting the animal on 30% carbon dioxide and short tracings were made at intervals during the exposure. At the end of 2 hours the carbon dioxide concentration was increased to approximately 40% and this was continued for another 2 hours. At the end of this time the dog was changed to breathing air and hyperventilated by means of a positive pressure respirator. Starting at the moment the change from high carbon dioxide to air was made, a continuous electrocardiogram was taken until the dog died or the tracing appeared to be returning to normal. With this procedure 7 of 10 dogs died in cardiac arrest within 2½–10 minutes after changing from carbon dioxide to air. Irregularities in cardiac rhythm, usually premature systoles, appeared in 30 seconds to 4½ minutes. Marked electrocardiographic changes were also noted in the case of the 3 dogs that survived. Conspicuous among these changes were premature systoles, broadening of the R wave, and partial atrio-ventricular block.

Differentiation of black- and red-dispersing factors from the brain of the fiddler crab, *Uca*. FRANK A. BROWN, JR., AND MILTON FINGERMAN. *Dept. of Biological Sciences, Northwestern Univ., Evanston, Ill.*

Brains of *Uca pugilator* were dissected out, smeared to a thin film on a coverslip, and allowed to dry completely. The coverslip was then shaken for 5 minutes in a test tube containing absolute isopropyl alcohol. The alcohol was decanted and evaporated. Sufficient sea water was then added to the evaporating dish to give a concentration of one brain/0.15 ml. The same volume of sea water was then used to extract the original brain smear once more. The resulting alcohol-soluble and alcohol-insoluble fractions were assayed upon eyestalkless *Uca pugilator* whose black and red chromatophores contained concentrated pigment. Each assay animal was injected with 0.05 ml. of

extract and the average chromatophore state observed during the succeeding 2 hours. The alcohol-soluble fraction had much black-dispersing activity and little red-dispersing activity. The alcohol-insoluble fraction had less black-dispersing activity and more red-dispersing activity than the former. It was concluded, therefore, that the red-dispersing and black-dispersing actions of extracts of the brain are due to two separate principles.

Acute experiments on the disposition of inhaled $C^{14}O_2$. AUSTIN M. BRUES AND AGNES N. STROUD.* *Div. of Biological and Med. Research, Argonne Natl. Lab., Chicago, Ill.*

By means of tracheal cannulation, rats and mice were permitted to inhale $C^{14}O_2$ at a relatively constant concentration in air for 30–60 minutes. Measurements were made of the clearance of the radioactive gas from air into the animals, and of the rate of respiratory loss during the period following cessation of exposure, when room air was being inhaled. The results indicate that the rapidly exchanging carbon compartment is composed of at least two portions, of which only one (presumably blood and soft tissue bicarbonate) is directly in equilibrium with alveolar air. Bone carbonate is probably the major compartment involved. As a result of this complexity, the rate of loss of C^{14} after the exposure period is much less than the rate of uptake during exposure. By assuming a three-component system, which is the simplest that can be involved, the findings are closely approximated by means of a model.

Demonstration of differential effects on pulmonary and systemic arterial pressure induced by breathing low-oxygen mixtures. HOWARD B. BURCHELL AND EARL H. WOOD, *Mayo Fndn., Rochester, Minn.*

In 3 of 4 patients with a patent ductus arteriosus and associated pulmonary hypertension, transient reversals in direction of flow through the ductus could be induced by the breathing of low-oxygen mixtures. The studies afford further evidence that the pulmonary resistance in unanesthetized human beings varies in the opposite direction to the oxygen content of the inspired gas. In the first subject, age 36, the pulmonary and systemic arterial pressures were approximately equal. When 100% oxygen was breathed, the oxygen content of right radial arterial blood was identical to that of femoral arterial blood. When air, 12% oxygen and 8% oxygen were breathed, however, there was a progressively greater difference between the femoral and radial arterial blood amounting to 1.0, 3.6, and 4.0 volumes % less oxygen in femoral than in radial arterial blood, respectively. Studies on two other occasions yielded similar results. The second patient, 3½ years old, had a pulmonary arterial pressure of 70/47 mm. Hg. The oxygen content of the right radial and femoral arterial samples were, respectively: breathing 100% oxygen, 15.8 and 16.1; breathing air, 13.4 and 13.5; breathing 10% oxygen, 2.8 and 1.3 volumes %. In this instance, after 5 minutes of breathing 10% oxygen, there was practically complete exclusion of the lungs from the circulation, the oxygen content of arterial blood reaching nearly the same very low oxygen content as pulmonary arterial blood. The third patient, age 21, had a very slight reversal of flow through the ductus induced by the breathing of 12% oxygen.

Membrane potentials of the cat auricle and their response to parasympathomimetic drugs. A. S. V. BURGEN AND K. G. TERROUX.* *Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada.*

The membrane resting and action potentials of the isolated cat auricle are readily recorded by micro-electrodes inserted through the endocardial surface. The mean resting potential measured in 276 fibres was 59 mv. and the mean action potential 61 mv. Although the action potential frequently reverses the membrane potential, in many fibres whose resting potentials were within the usual limits and which were apparently in good condition, reversal did not occur. Small amounts (10^{-8}, 10^{-7} gm/ml.) of acetylcholine and carbamylcholine produced striking changes in the action potential. Its rate of rise and especially of fall were greatly increased and the total duration of the deflection reduced. When the amount of drug was increased further, the height of the action potential progressively declined, but even with the highest concentration used (5×10^{-5} gm/ml.), conduction was well maintained. There was no evidence that these compounds decreased the resting potential. These effects were rapidly and completely reversed by atropine.

Permeability of the urinary bladder to radioactive mercury, calcium and sulfur. L. E. BURGESS,* D. T. ROLFE, P. F. HAHN, E. E. HOLMES* AND E. L. CAROTHERS.* *Dept. of Physiology and Cancer Research Lab. Meharry Med. College, Nashville, Tenn.*

We have previously shown (*Federation Proc.* 9: 20, 1950), that the urinary bladder of dogs was permeable to radioactive phosphorus and mercury. In the current experiments we have investigated the permeability of the urinary bladder to concentrations of Hg^{203} as $HgCl_2$ (20 mg/cc. and 10 mg/cc.), Ca^{45} as $CaCl_2$ (18.4 mg/cc. and 9.2 mg/cc.) and S^{35} as Na_2SO_4 (0.0500 mg/cc. and 0.0244 mg/cc.) instilled into the urinary bladder according to the technic previously described. The analyses for the radioactive isotopes were made at

hourly intervals for 5 hours on samples of urine simultaneously collected from both ureters. The blood showed a rapid uptake of Hg^{203} in both concentrations. There seemed to be a slightly greater uptake of Hg^{203} with higher concentration. The uptake of Ca^{45} by the blood reached its peak at the first hour in both concentrations. The peak of higher concentration Ca^{45} was much greater than that at lower concentration. There was rapid uptake of S^{35} in the blood at high concentration until the third hour. In low concentration, S^{35} uptake was gradual, reaching its peak at the third hour and remaining nearly constant until the fifth hour. The amount of excretion of Hg^{203}, Ca^{45} and S^{35} by the kidneys as revealed by analysis of ureteral urine, increased with the higher concentrations of isotopes instilled into the bladder.

Protective action of some sulfur-containing and sulfur-free compounds against x-ray damage in bacteria. W. T. BURNETT,* G. E. STAPLETON* AND A. HOLLAENDER. *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

A detailed survey designed to discover additional substances which will materially reduce the degree of damage produced in biological material by ionizing radiations has shown that a wide variety of chemical compounds have the ability to modify quantitatively the lethal action of x-radiation on *Escherichia coli*. Compounds which have been found to afford protective action to this microorganism include 2,3-dimercaptopropanol (BAL), methionine, mercaptosuccinic acid, glycerol, propylene glycol, triethylene glycol and certain derivatives of furfural. Hollaender, Stapleton and Martin (*Nature*, in press) reported previously that pretreatment with cysteine and glutamic acid gives some protection. Cysteine, BAL and the glycols have been studied in detail. With the sulfur-containing compounds, it would appear that 0.02 M is a limiting concentration in either oxygen- or nitrogen-saturated suspensions. Moreover, protection with cysteine and BAL, alone or in combination up to 0.02 M, is proportional to the sulfhydryl content. The glycols showed a linear relationship between protective action and concentrations up to about 20%. Triethylene glycol, added to BAL at the 0.02 M level, gave additional protection which was proportional to the concentration, indicating that different mechanisms may account for the protection afforded by the glycols and by the sulfhydryl-containing compounds.

Prophylaxis of experimental renal malignant hypertension with crude and semipurified hog renins. RICHARD O. BURNS, JR.,* BESS G. OSGOOD* AND G. E. WAKERLIN. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Crude and semi-purified hog renins and control crude hog liver extract were injected intramuscularly daily for 3 months prior to simultaneous bilateral renal artery constriction and subsequently during survival for a maximum of one month. Six groups of dogs were studied: *group I*, untreated malignant hypertensive controls; *group II*, crude liver extract; *group III*, crude renin from whole kidney (2.0 DU/kg/day); *group IV*, semi-purified renin from whole kidney (10 DU/kg/day); *group V*, crude renin from renal cortex (2.5 DU/kg/day); *group VI*, semi-purified renin from cortex (10 DU/kg/day). Results were as follows:

Group	No. of Dogs	Protected against Hypert. %	Death %	Lesions %
I	13	0	0	0
II	3	0	33(?)	0
III	4	0	0	33
IV	4	25	25	67
V	17	30	55	100
VI	6	67	0	67

Protection against hypertension showed no correlation with the antirenin titer in *group IV*, but a high correlation in *groups V* and *VI*. No correlation was found between the antirenin titers and protection from death or arteriolonecrotic lesions. The evidence suggests that the non-renin fraction of renal cortex in the absence of medulla protects from death and arteriolonecrotic lesions.

Extreme hypercholesteremia following administration of cholic acid to the bile duct ligated rat. SANFORD O. BYERS AND MEYER FRIEDMAN. *Harold Brunn Inst. for Cardiovascular Research, Mount Zion Hosp., San Francisco, Calif.*

Ligation of the bile duct of the albino, Long-Evans, or Sherman strain rat results in a 200–400% increase in plasma cholesterol concentration within 72 hours, together with a 400–800% increase in bile acids. If the liver and its circulation remain intact, the cholesteremia is unaffected by starvation, diet, or abdominal surgery, including denervation of the liver. Oral administration of large amounts of cholic acid daily for 3 days, however, results in extreme hypercholesteremia. The data, expressed in mg.% cholesterol, follow. Before ligation: 48 (av. of 10 rats). After ligation: No cholic acid, 100–201 (range of 16 rats); 50 mg. cholic acid, 456 (av. of 15 rats); 100 mg. cholic acid, 602 (av. of 27 rats); 150 mg. cholic acid, 580 (av. of 26 rats); 200 mg. cholic acid, 709 (av. of 6 rats). Plasma bile acid concentrations at 72 hours after ligation ranged from 22 to 224 mg.%. Higher plasma cholesterol values tended to be associated with higher plasma bile acid values. It is suggested that accumulation of bile acid is

one of the causes of hypercholesteremia in the bile ligated rat.

Differential vagal responses as demonstrated by ECG. B. CALESNICK AND N. H. SMITH (introduced by M. E. MAXFIELD). *Dept. of Pharmacology, Hahnemann Med. College, Philadelphia, Pa.*

Healthy mongrel dogs were anesthetized with morphine sulfate and chlorobutanol and the vagosympathetic nerves were electrically stimulated with an inductorium. Continuous electrocardiograms, using a direct ink-writer, were taken of the animals during these investigations. After adequate controls, procaine HCl was administered by slow infusion at a constant rate of 1 mg/kg/min. The intact nerves were then alternately stimulated every minute after the start of the infusion. Invariably, the left vagal response was initially blocked and finally, when a total of 20 mg. of this drug was administered, it was observed that stimulation of either the right or left nerve produced apnea but failed to elicit the classical cardiovascular response. Acetylcholine was also blocked at this dosage. The pressor action of epinephrine was observed to be prolonged. The slow infusion of procaine HCl resulted in a slight fall in blood pressure; whereas, a single injection of the same dose produced a hypotension, bradycardia and apnea. The continuous electrocardiographic recordings during the slow infusion of procaine HCl also demonstrated the following: *1*) acetylcholine simulated left vagal stimulation; *2*) asphyxia resulted in an initial left vagal block; *3*) asphyxia prior to the procaine infusion terminated the studies in a respiratory death at approximately a dose of 10–15 mg/kg.

Use of a new respirometer in studying the effects of adrenocortical extract on total metabolism. THOMAS B. CALHOON* AND CLIFFORD A. ANGERER. *Dept. of Physiology, Ohio State Univ., Columbus.*

A new type of respirometer has been developed in this laboratory for the measurement of the oxygen consumption of aquatic and amphibious animals. It has several advantages over the Winkler technique: determinations can be made, continuously, for several hours; a better linear relation can be produced between oxygen consumption and time; it allows a direct reading of the oxygen consumed. The principle is based upon making determinations of volume change at an original pressure level. Any decrease in pressure is compensated by a decrease in total volume of the apparatus, which is effected by an appropriate movement of calibrated syringes. The apparatus consists of a 500-ml. animal chamber. There are 3 outlets from this chamber. The first is a sidearm containing a CO_2 absorber. The second leads to an index-marked capillary filled with an indicator solution. This tube is closed by a compensatory flask attached to its distal end. The third outlet leads to the measuring device which consists of an air-tight syringe calibrated in 1/100 of a ml. A comparison of the Winkler method and this respirometer was made using the oxygen consumption of goldfish. Using this apparatus, we have reported a series of investigations on the effects of various dosages of sesame oil and adrenocortical extract on the oxygen consumption of goldfish. (*Anat. Rev.* Suppl. 105: 142, 1949; *Endocrinology* 46: 327, 1950; *Federation Proc.* 9: 21, 1950). Further studies are in progress on the effects of various dosages of adrenocortical extract on the oxygen consumption of frogs. These results support our previous suggestions that adrenocortical extract in the dosages used depresses the total metabolism of coldblooded animals.

New operational procedures in the study of the cerebral cortex. BERRY CAMPBELL. *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Methodology and theory are discussed and illustrated whereby a more operational approach may be made to the study of the cerebral cortex. A re-evaluation of the problem of the laminar structure has shown the inadequacy of the present methods in that they *1*) necessitate logical assumptions being made as to layer structure prior to the derivation of data, and *2*) rely upon verbal and subjective description. It is shown to be possible to present quantitative expression of the cortical pattern by plotting as continuous curves various well chosen parameters (cell frequency, cellular area, myelin density etc.) against depth from surface to base of the cortex. Similar techniques applied to the problem of areal variation of structure have shown the inadequacy of the parcellation concept. Reinterpretation of data from the literature and information newly derived has lead to the formulation of a cortical map of completely different nature and import than those hitherto drawn. As a basis, the cortex is considered a continuum with characteristic architectural gradients distributed in a systematic and meaningful pattern. The maxima of the structure alteration gradients are at the central fissure, the edge of the striate area, and the rhinal fissure. Large areas of minimal change are found in the frontal lobe, the parieto-temporal region, and within the striate area. Application of these operational attacks are shown to the problems presented by comparison of one brain with another (relative to Gaussian variation), of the brains of psychotic with the sane, one age with another, and of one species with another.

Urinary 17-ketosteroids in chronic liver disease. A. CANTAROW, K. E. PASCHKIS, T. L. WILLIAMS* AND W. P. HAVENS, JR.* *Jefferson Med. College, Philadelphia, Pa.*

Data have been obtained on the daily urinary excretion of free and total neutral 17-ketosteroids in normal subjects and patients with hepatic disease before and for 7 days after a single injection of 200 mg. testosterone propionate. The pre-injection values for total 17-ketosteroid were lower and the proportion existing in the free state was higher in patients with liver disease than in normal subjects. The calculated recovery, as 17-ketosteroid, of 200 mg. testosterone propionate was 12.8–59.6% in the normal subjects and 9.5–40.9% in those with liver disease. The maximum 24-hour excretion in free form was much higher in the latter group (1.5–10.9 mg., as compared to 0.6–0.9 mg. in the controls), as was the percentage of the total excreted in the free state (7.7–36.2%, as compared to 1.5–3.4% in the controls). These data suggest that conjugation of metabolites of testosterone is impaired in subjects with chronic liver disease.

Decomposition products of irradiation of purines and pyrimidines. ATTILIO CANZANELLI AND DAVID RAPPORT. *Dept. of Physiology, Tufts College Med. School, Boston, Mass.*

Adenosine-3-phosphate, adenine, hypoxanthine, uracil and thymine have been irradiated in phosphate buffered solution at pH 7.4 in concentration of 5×10^{-5} M with a G.E. low intensity ultraviolet germicidal lamp for a period of about 18 hours. At this time, as we have previously shown, the above substances have completely lost their specific ultraviolet absorption spectra. Under these conditions there is approximately a mol of ammonia produced for each mol of adenine and adenosine-3-phosphate irradiated. However, most of this probably does not result from deamination, since the oxypurine hypoxanthine yields almost as much ammonia. Irradiation of the pyrimidines, on the other hand, gives rise to a very small ammonia production, from which it may be inferred that at least the greater part of the ammonia derived from the purine nucleus comes from the imidazole ring. A relatively small amount of urea is produced from all of the substances; more from thymine than from the others. Additional substances which are neither urea nor allantoin, but which react with diacetyl monoxime, are produced in variable quantities. These chromogens have not yet been identified.

Further evidence of an inverse relation between body weight and fecundity in rats. A. J. CARLSON AND F. HOELZEL.* *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

This report concerns the effect of segregation and isolation on gain in weight and on the conception time of littermate rats mated when 97 to 138 (av. 120) days old. The number of days between mating and casting of first litters, less a gestation period of 22 days, is regarded as conception time. As in previous fertility studies (*Federation Proc.* 9: 354, 1950; *Am. J. Physiol.* 163: 703, 1950), rats raised on three diets were used but the data obtained with our richest diet were too meager to be of any value as the reproduction of rats on this diet ceased completely during the present study. Data were easily secured on 10 segregated and 10 isolated littermate pairs of rats on our bulky but otherwise rich diet and on our high-cereal, least rich or relatively poor diet. The isolated rats on both diets gained more weight and had longer conception periods than their segregated littermates but the increases were greater in the rats on the high-cereal diet. That is, the isolated rats on the bulky diet gained only 11% more weight (P approx. 0.2) and had a conception time only 25% longer (P 0.5–0.6) than their segregated littermates while the isolated rats on the high-cereal diet gained 21% more weight ($P < 0.01$) and had a conception time 141% longer ($P < 0.05$) than their segregated littermates. The increase in conception time therefore seems to be partly proportional to the gain in weight.

Dependence of steady-state rate of oxygen metabolism on frequency of conducted impulses in peripheral nerve. F. D. CARLSON* AND F. BRINK. *Jenkins Labs. of Biophysics, Johns Hopkins Univ., Baltimore, Md.*

The increase in the steady-state rate of oxygen uptake accompanying the conduction of impulses in frog (*R. pipiens*) sciatic nerve has been determined with the flow respirometer (CARLSON, BRINK, BRONK. *R.S.I.* 21: 923, 1950). The increase in the steady-state uptake of oxygen approaches a limiting value with increasing frequency. On the basis of threshold and action potential area correlations it is concluded that the observed dependence of the steady state of oxygen uptake on stimulation frequency is associated with the activity of the A-α fibers. The increase in the steady-state rates of oxygen uptake ±a.d. at 20°C. are: 0.12 ± .04, 0.24 ± .11, 0.43 ± .10, 0.77 ± .16, 1.10 ± .24, 1.07 ± .07 μM per gram-hour for frequencies of: 4.2, 8.9, 16.8, 44.0, 95.0, 195.0 conducted volleys per second, respectively. The curve of Feng and Hill (*Proc. Roy. Soc. London s. B* 113: 356, 1933) yields the following values for the increase in steady-state heat production (R. esc. at 19°C.): 10, 16, 26, 32, 36, 39, 40 micro-calories per gram-second for frequencies of 10, 20, 40, 60, 80, 100, 120 shocks per second, respectively. Comparison plots of increased steady-state oxygen uptake and increased steady-state heat production versus frequency of stimulation agree well within the limits of experimental error although the heat production calculated from the observed oxygen uptakes is about 15% low, based on a

calorific equivalent of 4.85 calories per cc. of oxygen.

Functional changes in the feline bladder after interruption of the parasympathetic innervation. FRANK G. CARPENTER (introduced by MAGNUS I. GREGERSEN). *Dept. of Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

The successive introduction of fluid into the bladders of 6 cats under bulbocapnine produced small increases in intravesical pressure. At a volume of 40 cc. the pressure was 5–6 cm. H_2O and sustained detrusor contraction occurred. Comparison of these curves with 22 obtained under light pentobarbital anesthesia shows that at comparable bladder volumes the same intravesical pressures were found. In these animals detrusor contraction occurred at 60–130 cc. volume and about 10 cm. H_2O pressure. After sacral root infiltration with 3% procaine, the volume-pressure curves of 7 cats anesthetized with pentobarbital were essentially the same as those obtained on normal bladders. Detrusor contraction did not occur. Immediately after sacral root section (13 cats) the volume-pressure curves were the same as those found following procaine infiltration. Ten days after the sacral roots were cut the bladder capacity at 15 cm. H_2O intravesical pressure was more than twice that measured after procane infiltration of the sacral roots. This increased capacity of the bladder remained unchanged as long as 46 days after operation. After bilateral pelvic nerve section the bladder capacity showed first an increase (5 cats, 7–21 days), then a decrease (4 cats, 40–67 days) below the control value. Autonomous contractions were seen 3 or more weeks after bilateral section of the sacral roots or after bilateral section of the pelvic nerves. In the latter preparations these contractions were large.

Increases in rat adrenal cholesterol produced by dietary fats and oils. K. K. CARROLL (introduced by J. A. F. STEVENSON). *Dept. of Med. Research, Univ. of Western Ontario, London, Ontario, Canada.*

Various fats and oils were fed to groups of rats as 25% of their diet for a month. Oils such as rape oil and turnip seed oil which contain high proportions of erucic acid increased the cholesterol content of the rats' adrenals to 3–5 times that of rats fed on fox chow. Other fats and oils caused much smaller increases in adrenal cholesterol. Whereas the erucic acid oils increased the adrenal size up to 1½ that of normals, other fats and oils only slightly increased or in most cases decreased the adrenal size. The nature of the chemical in rape oil responsible for these effects and the physiological activity of such adrenals will be discussed.

Effect of feeding thiourea on morphology of thyroid and adrenals in castrated mice of the C_3H strain. CARMEN B. CASAS AND ENRIQUE KOPPISCH (introduced by ROGER M. REINECKE). *Depts. of Physiology and Pharmacology and Pathology, Univ. of Puerto Rico School of Medicine, San Juan.*

The inbred strain of mice, C_3H, develops tumors of the adrenal glands following castration. The adrenals atrophy in these same mice if fed thiourea. Mice of both sexes of the C_3H strain were started on 0.3% thiourea in the Rockland Rat Diet fed *ad libitum* starting immediately after they had been castrated. After from 3–7 months on this drug the animals' thyroid glands showed hyperplastic changes with diminished colloid and enlarged acinar cells, and their adrenals presented evidence of diffuse hyperplasia with a tendency to form adenomas. The females never showed signs of vaginal stimulation, as is true of the ovariectomized animal on a regular stock diet. The weight of the animals was definitely less than that of castrated controls not receiving the drug.

Inactivation of Cypridina luciferase by alcohols. AURIN M. CHASE. *Physiological Labs., Princeton Univ., Princeton, N. J.*

The luminescent reaction of luciferin and luciferase, extracted and partially purified from the ostracod crustacean, Cypridina hilgendorfii, was measured quantitatively (at room temperature and pH 6.8) in the presence of various concentrations of the straight-chain alcohols from methyl to hexyl. (Solubility difficulties prevented the use of alcohols higher than the latter.) It was found that all the alcohols studied had a completely reversible, inhibiting effect upon the reaction; the effect increasing with the chain length of the alcohol. The site of action appeared to be the enzyme, as indicated by the fact that the first order velocity constant of the reaction decreased but the total light emitted remained relatively constant. Hexyl alcohol was about one hundred times as effective in producing a given amount of inactivation of luciferase as was methyl alcohol. For the 6 alcohols studied, each additional carbon atom in the chain resulted in an increase in effectiveness of two to three times; a result very similar, quantitatively, to that which has been obsevred for alcohol anesthesia *in vivo*.

Fluorimetric determination of steroids in minute quantities. CHIADAO CHEN (introduced by W. B. BRADLEY). *Dept. of Exptl. Medicine. Northwestern Univ. Med. School, Chicago, Ill.*

In the course of studies of steroid metabolism it was found desirable to devise a method by which

minute amounts of hydroxysteroids could be determined quantitatively. Hydroxysteroids were treated with 7-methoxy-cumarin-3-carboxyl-chloride and pyridine in benzene solution. The reaction's product was taken up in benzene and washed with dilute hydrochloric acid, dilute sodium bicarbonate and water to remove pyridine and excess acid chloride or its corresponding carboxylic acid. An aliquot of the benzene solution was diluted with methanol and read with the aid of a Cenco-Friedemann-Liebeck fluorimeter. 0.05 gamma of cholesterol, compound S, testosterone, etc. could be determined quantitatively.

Effect on the electrocorticogram of local topical application of acetylcholine chloride to the human cerebral cortex. J. G. CHUSID* AND C. G. DE GUTIÉRREZ-MAHONEY. *Neurological Div., St. Vincent's Hosp., New York City.*

The electrocorticograms of several patients under general anesthesia were studied before and after the local topical application of acetylcholine chloride in 10% or 20% solutions to the cerebral cortex. High amplitude spike discharges were sometimes recorded from the local area as early as 3–6 minutes following application of the acetylcholine chloride solution. Initially the spike discharges occurred singly and at random, later in short periodic bursts. On occasion, repeated application of acetylcholine chloride solution produced typical spike discharges when the initial single application had been ineffectual. Most areas tested were in a frontal lobe close to a tumor site; in one patient with a frontal lobe tumor (local anesthesia), application of 20% acetylcholine chloride solution to the exposed ipsilateral parietal cortex produced typical local (parietal) spike discharges. However, some areas of cortex either adjacent to or distant from a pathological site did not exhibit the spike discharge phenomenon after repeated or prolonged application of 10% or 20% acetylcholine chloride solution. Depression of the electrical activity of the cortex prior to the appearance of spike discharges, as described by others for the cat, was not observed in this group of patients. No systemic effects which could be attributed to the acetylcholine chloride were noted.

Transmission and penetration of thermal radiation into skin. CARL CLARK (introduced by J. O. PINKSTON). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

The penetration of radiation into and emission from the skin determine the radiation exchange of the organism with its environment. These in turn are affected by the chemical and physical properties of skin. A review of available data on the penetration of radiations from short x-rays to 16 micron infrared is presented and correlated with the chemical and physical properties of skin. Recent measurements made in this laboratory on the penetration of infrared radiation into skin are presented, with emphasis on the effects of scattering as measured by an angle scanning device.

Cessation of spontaneous walking elicited by stimulation of various subcortical structures in unanesthetized dog. GEORGE CLARK, S. E. GOLDBERG* AND M. G. GOLDSBAND.* *Dept. of Anatomy, Chicago Med. School, Chicago, Ill.*

Electrodes were implanted aseptically at various subcortical locations in a series of dogs, and the animals were tested on the day following operation when they were awake and alert. Stimulation (60 cycle sine wave) at most of these points produced a cessation of spontaneous walking with no apparent loss of postural tone. Often there was some movement, such as turning the head, associated with this arrest. Supraliminal stimulation elicited various complicated clonic movements outlasting the stimulus. These were often so intense and generalized as to be best described as convulsions. The active points were widely scattered in the white matter and included internal capsule, corona radiata, fornix, corpus callosum, etc. Several were located near the border of the caudate nucleus but arrest was not elicited from points centrally located in the caudate nucleus. A similar (?) cessation of spontaneous walking in the cat was termed 'suppression' by Ward and the 'arrest reaction' by Hunter and Jasper. It is difficult to see how either of these terms can be applied to the response we have observed.

Effect of ultraviolet radiation upon incidence of spontaneous mammary tumors in mice. JANET HOWELL CLARK. *Dept. of Biology, Univ. of Rochester, Rochester, N. Y.*

Exposure of C3H mice to low intensity ultraviolet radiation resulted in earlier development of spontaneous mammary tumors. Three experiments were carried out. In the first experiment *group I* lived entirely in the dark; *group II* lived in the dark but were exposed to ultraviolet radiation 3 times a week until the total dose reached 150 erythema units; *group III* lived in controlled artificial daylight for 12 hours a day. At the age of 300 days 50% of the ultraviolet group had developed tumors, 33.3% of the dark group and none of the light group. In the second experiment the group exposed to ultraviolet radiation lived under the same conditions as the light group and were given exposures up to 200 erythema units. Under these conditions the effect of the ultraviolet radiation was less marked. The average age of tumor incidence was 423 days in the dark group, 408 days in the light group and 340 days in the light group exposed to ultraviolet radiation. In the third experiment the conditions in the first experiment were duplicated. These experiments are still in

progress but at the age of 360 days 74% of the ultraviolet group had developed tumors, 57% of the dark group and 46% of the light group. It seems, therefore, that exposure to low intensity ultraviolet radiation tends to hasten the development of spontaneous mammary tumors in C3H mice. (Supported by a grant from the Jane Coffin Childs Memorial Fund.)

Rapid depletion of ionic calcium by circulating blood of animals over cationic exchange resins. LELAND C. CLARK, JR.,* FRANK GOLLAN AND RICHARD SMITH.* *Fels Research Inst., Antioch College, Yellow Springs, Ohio.*

In a search for a method to render blood incoagulable *in vivo* without the use of anticoagulants, experiments were undertaken to determine the physiological effects of acute removal of ionizable calcium from the circulating blood. For this purpose venous blood of dogs was pumped at about 500–700 cc. per minute through an extracorporeal circuit in which a chamber containing 300 ml. of cationic resin, buffered at pH 7.2 was inserted. Dowex 50 (Dow Chemical Co.) or IRC-50 (Rhom and Haas Co.) was activated by treating 24 hours with 10 volumes of 10% sodium hydroxide, washing with 3 volumes of water, treating for 12 hours with 3 volumes of 4% sodium phosphate buffer (pH 7.2), washing with dilute buffer until the effluent has a pH of 7.2, and finally with isotonic saline. The resin was supported by fine quartz gravel which served as a filter. The most apparent physiological effects of this rapid removal of ionic calcium consisted of incoagulability of the blood, changes in the electrocardiogram, and tetanic convulsions. These changes could be immediately reversed by the infusion of calcium chloride. In this way it is possible to maintain the circulating blood inside the animal at its normal clotting time, while that in the outside circuit is incoagulable. This procedure may lend itself to experiments involving circulation of the blood outside the body, such as in the use of mechanical heart-lung apparatuses. This procedure suggests the possible use of other cationic and anionic resins for the rapid removal of other specific blood constituents.

Secretin inactivation by serum. W. A. CLAY (introduced by W. J. SNAPE). *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

The secretin-inactivating action of serum, described by Greengard, Stein, and Ivy, was tested on secretin preparations produced at different stages in the procedure of Friedman and Thomas. Potency of secretin preparations and the degree of their inactivation was tested on dogs under sodium pentobarbital (Nembutal) anesthesia. It was found that the crude acetone-precipitated methanol extract was inactivated by incubation with human and animal serum. When this acetone precipitate was dissolved in water and reprecipitated with trichloracetic acid it showed little or no inactivation on incubation with serum. Inactivation by serum, however, was still demonstrated if before incubation with the serum the trichloracetic acid-precipitated fraction was first frozen or lyophilized. Treatment of secretin with trichloracetic acid may protect a susceptible linkage, the breaking of which results in loss of secretin activity. Evidence was obtained that this protection was not due to destruction of the serum inactivators by trichloracetic acid. The means whereby the protective effect of treating the secretin with trichloracetic acid is nullified by simple freezing or freeze-drying remains to be explained.

Production of periods of leukocytosis and tolerance by consecutive oral administrations of different bacterial pyrogens. CARMINE D. CLEMENTE (introduced by RUTH RHINES). *Dept. of Anatomy, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Several bacterial pyrogens (from a Pseudomonus species, Proteus vulgaris, and Bacillus subtilis) induced periods of significant leukocytosis when administered orally to the rat. In each instance the animal developed tolerance to continuous administration of the pyrogen as evidenced by return of its leukocyte count to the control level. The present experiments aimed to determine whether the tolerance effect is general or specific for pyrogens from different organisms. Each rat received approximately one gamma of a Pseudomonus pyrogen daily in the drinking water. After 2–5 days the total leukocyte count increased from an average control value of approximately 16,000 cells/cu.mm. to 29,000 or 35,000 cells/cu.mm. After 7–10 days the tolerance effect was demonstrated by the return of the leukocyte count to the control level. Administration of the same pyrogenic extract was continued orally for 5–7 days after tolerance had developed, and the white count remained at control levels. A different pyrogen (Proteus vulgaris or Bacillus subtilis) was then substituted in the drinking water. Each rat showed a secondary leukocytosis in which the count sometimes exceeded 40,000 cells/cu.mm. In the course of time a secondary period of tolerance developed. The pyrogen from Proteus vulgaris produced the most prolonged periods of leukocytosis. In some of the animals the white count did not return to control level for 20 days and during this period there were several peaks. (Aided by a grant from Baxter Laboratories, Inc.)

Studies on mitral stenosis produced in the dog. GEORGE H. A. CLOWES, JR. (introduced by CHARLES H. BEST). *Banting and Best Dept. of Med. Research, Univ. of Toronto, Ontario, Canada.*

Mitral stenosis has been produced in dogs by partially approximating the leaflets of the exposed mitral valve with sutures. This was accomplished using an extracorporeal pump to by-pass the left side of the heart during the operative procedure. Blood was drawn from the pulmonary veins and returned under pressure to the arterial system. While the left heart was open, the coronary arteries, and indeed the whole animal, were perfused with blood oxygenated in its own lungs. The valvular stenosis was in most instances progressive. Vegetations formed postoperatively on the mitral valve near the sutures. As healing took place, the mitral opening became increasingly narrowed. Deaths occurred at periods ranging from 12 hours to 3 weeks after operation. The length of time depended in part upon the degree of stenosis produced. These animals exhibited acute pulmonary edema, and in some instances, right heart failure with swollen liver and ascites. Observations are being made on the progress of this condition by measuring cardiac output and by comparing pulmonary arterial pressure with left auricular pressure. Data obtained both at rest and during exercise will be presented. It is believed that this technique for the production of definite degrees of mitral stenosis in the dog may be of value in further elucidating the mechanisms of pulmonary edema and failure of the right heart.

Kinetics of increased oxygen consumption of activity in frog nerve. C. M. CONNELLY (introduced by P. W. DAVIES). *Jenkins Labs. of Biophysics, Johns Hopkins Univ., Baltimore, Md.*

The time course of change in rate of oxygen utilization (Q_{O_2}) by active nerve has been examined to determine parameters characteristic of the metabolic reaction pattern in nerve, and the relationship of each parameter to frequency of conducted impulses. Using an oxygen cathode in direct contact with nerve, it has been found that the increase in Q_{O_2} is of the form $(1 - e^{-k_1 t})$ during activity and $e^{-k_2 t}$ during recovery. Neither k_1 nor k_2 is measurably dependent on frequency in the range 20 to 50 impulses/second. Reciprocals of k_1 and k_2 fall within 4–8 and 10–20 minutes, respectively, at 20°C. Limits of technique do not preclude possible occurrence of components of respiration with time constants 1.5 minutes or less. By other experiments, initial change in Q_{O_2} during activity has been measured at different frequencies. The time rate of change of Q_{O_2}, constant for some tens of seconds, does not bear the same relationship to frequency as does the final steady-state Q_{O_2}, (see CARLSON and BRINK, this meeting) nor is it linear with frequency. It approaches a limiting value, or saturates, at 300 to 400/second, just as does the rate of initial heat production (HILL). Since these two variables show similar frequency dependence at a time early in the course of metabolic change, it is suggested that both initial heat and initial changes in Q_{O_2} reveal the kinetic properties of reaction steps very close to the conduction process.

Studies on mechanism of bile formation. DONALD L. COOK,* W. E. HAMBOURGER AND D. M. GREEN. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

Clearance techniques similar to those used in renal function studies were applied to the study of bile formation in the anesthetized dog. Measurements of the biliary clearance of a variety of substances, both endogenous and exogenous, at constant and at increasing plasma concentrations, indicated two distinct mechanisms for bile formation. Water, electrolytes and some crystalloids of low molecular weight such as creatinine, were cleared at low and comparable rates by a process suggestive of filtration. The clearance values for more complex crystalloids such as bromsulphalein and *p*-aminohippurate were 20–50 times greater and were indicative of a secretory mechanism. The clearance of 'filtered' substances was increased by hydrocholeretics, while that of 'secreted' substances was unaffected or decreased.

Cerebellar component of convulsive manifestations. W. H. COOK* AND G. W. STAVRAKY. *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.*

In a series of 26 cats, aseptic removal of various regions of the cerebellum was carried out and after complete recovery of the animals convulsions were induced by means of intravenous injections of metrazol or of acetylcholine. Removal of half the cerebellum caused the cats, post-operatively, to lie on the side of the lesion and sometimes to roll to that side. Compensation progressed, until one year after the operation tremor and ipsilateral ataxia were the only outstanding residual symptoms. At that time injections of metrazol or of acetylcholine reproduced during the convulsion the original syndrome of cerebellar removal—the animals falling, rolling, staggering and circling to the operated side. Following decortication of half of the cerebellum, the cats were ataxic on the side of the lesion but leaned and staggered to the opposite side for 1 to 3 weeks. After complete recovery from the latter symptoms (leaning and staggering), metrazol and acetylcholine caused in these animals convulsions during which the cats fell, rolled, staggered and circled to the side opposite to the cerebellar lesion. Partial ablation of various regions of the cerebellar cortex gave essentially similar results but the asymmetrical manifestations were less pronounced. Lesions in the cerebellar nuclei placed with the Horsley-

Clarke stereotaxic instrument also caused the animals to fall, roll, stagger and circle during chemically induced convulsions, the direction of these manifestations varying with different lesions. A histological study of the exact topography of the various cerebellar ablations is in progress at the present time.

Acetaldehyde blood levels in response to tetraethylthiuram disulphide (Antabuse) and anesthetic agents. BETTY M. COOPER,* C. L. CLARK,* L. MISURACA,* H. C. SLOCUM* AND CHARLES R. ALLEN. *Dept. of Anesthesiology, Univ. of Texas Med. Branch, Galveston, Tex.*

Rabbits were given tetraethylthiuram disulphide by stomach tube in doses of 0.5 gm/kg. body weight. Fourteen hours later they were subjected to a period of anesthesia during which the effect of the anesthetic drug on acetaldehyde blood concentration was determined. The anesthetic agents tested were diethyl ether, divinyl ether, chloroform, nitrous oxide, cyclopropane, sodium pentothal, procaine and avertin.

Histo-physiology of teleostean physoclistus swimbladder. D. EUGENE COPELAND (introduced by JOHN R. MURLIN). *Arnold Lab., Brown Univ., Providence, R. I.*

The secretory and absorptive mechanism of the swimbladder of *Fundulus heteroclitus* was stimulated by deflation and overinflation, respectively. After stimulation, gas analyses showed the following maximal ranges: oxygen, 82–0%; carbon dioxide, 23.2–0%; nitrogen, 8.5–100%. The former was found in fish recovering from deflation, the latter recovering from overinflation. Carbonic anhydrase determinations of the secretory epithelium and the double rete showed practically no change per unit weight of tissue in recovery from repeated deflations, a slight decrease being noted in the epithelium. However, the total carbonic anhydrase increases as the result of approximately a triple hypertrophy of the gland. Cytologically, mitochondria or Golgi apparatus could not be demonstrated in the secretory epithelium and the cytoplasm was flocculent and slightly acidophilic by routine histological technique. A very marked correlation between glycogen in the epithelium and activity was found, glycogen being depleted on recovery from deflation and massively stored on recovery from overinflation. Analyses on *Perca flavescens* failed to confirm the report of Meesters and Nagel (*Ztschr. f. vergl. Physiol.* 21: 646, 1934) that *Perca fluviatilis* can produce 80% carbon dioxide during active inflation, the maximum observed being 7%. Preliminary results with pure gases substituted in the bladder indicate that oxygen facilitates the secretory mechanism more than does carbon dioxide or nitrogen. *Fundulus* cannot secrete gas beyond an ambient pressure of 4 atmospheres. The above results support the theory that secretion of gases can be accounted for by ordinary laws of blood dissociation under suitable pH control, it being assumed that the glycogen is metabolized either to CO_2 or to an intermediate organic acid.

Gelation phase of blood coagulation. ALFRED LEWIN COPLEY. *Dept. of Medicine, New York Med. College, New York City.*

The existence of the gelation or third phase of blood coagulation was postulated (COPLEY, A. L., Abstracts of Communications, XVIII International Physiological Congress, Copenhagen, August 15–18, 1950, p. 165) on the basis of anomalous findings of decreased coagulability (hypocoagulability), as measured by a slight modification of the blood-saline coagulation time test (COPLEY, A. L. AND R. B. HOULIHAN. *Science* 100: 505, 1944. COPLEY, A. L. *Science* 101: 436, 1945), and normal prothrombin time. Conversely, normal blood coagulability or increased coagulability (hypercoagulability), and marked prolongation of prothrombin time, as induced by Dicumarol and Tromexan in rabbits, were also found. The latter incongruence of blood coagulability and prothrombin activity does not permit the designation of the chemical hemorrhagic agent Dicumarol as a true anticoagulant. Heparin inhibits, both *in vitro* and *in vivo*, the gelation phase and therefore is regarded as a true anticoagulant. On the other hand, total body x-irradiation, a physical hemorrhagic agent, applied in doses of 700 and 800 r in rabbits, only occasionally produced hypercoagulability and hypocoagulability, and did not affect prothrombin activity. Investigations, using model tests concerning the gelation phase, among which the paradoxic effect of calcium in inhibiting gelation in higher blood-saline concentrations, and in accelerating gelation in lower blood-saline concentrations, will be presented. (Work performed under Contract No. N7onr-40102 of the Office of Naval Research, Department of the Navy and Contract No. AT(30-1)-780 of the Atomic Energy Commission.)

Electro-potential changes during spontaneous activity of the human urinary bladder. E. L. COREY, W. H. BOYCE* AND S. A. VEST.* *Univ. of Virginia Med. School, Charlottesville.*

Electro-myograms representing spontaneous activity of the normal, human urinary bladder were obtained by means of a special electrode-carrier in contact with the bladder wall, in conjunction with a Grass electroencephalograph, suitably filtered. Tracings secured from 12 subjects (7 females; 5 males) without demonstrable urinary tract pathology were selected as representative of normal bladder activity. Electro-potential changes were found to be quite constant in rate (av. 2.3/

second), duration (av. 4.7 second), and peak EMF generated (av. 0.357 mv.). The wave form produced on the records was found to be composed of 2 major components of opposite polarity, which we have termed 'A' and 'B' waves. In certain subjects, a small precursory deflection ('a' wave) occurred, and certain variations in the 'B' wave were occasionally found to be present in normal subjects. That the electro-potential changes recorded did, in fact, represent contractions of the bladder musculature was concluded from observations that typical deflections were produced *a*) when subjects attempted to void, *b*) in patients in whom pain accompanied spontaneous contraction, *c*) coincident with detectable (by palpation) movement of the electrode-carrier, and further *d*) from the fact that acceleration or suppression of activity (as shown by the record) occurred in pathological states in which cystic activity was known to be similarly affected. The advantages to the clinical urologist inherent in an electro-cystomyographic method for the determination of bladder motility are obvious.

Effect of exogenous thyroxine on iodine turnover in chick thyroid glands. R. L. CORNWALL* AND E. P. REINEKE. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Daily injections of 1 to 4 μg. of *d,l*-thyroxine per 100 gm. body weight for 2- and 4-week periods retarded uptake of I^{131} and total iodine content of chick thyroid glands in direct ratio to the thyroxine dose. Thyroid weight per unit body weight was suppressed almost equally (50%) at all thyroxine levels. I* turnover was inhibited more by 1 μg. doses than by 2 or 3 μg. Further inhibition was evident at the 4 μg. thyroxine level. Apparently daily thyroxine secretion rates of control animals, calculated from percentage turnover of I* between 48 and 96 hours and total thyroid iodine values, were threefold greater than reported determinations using the thiouracil technique. The secretion rate of chicks receiving the 1 μg. thyroxine supplement was inhibited more than in groups given 2 and 3 μg. doses. Inactivity of the thyroids in the former group is indicated by nearly maximal suppression of gland weight, greater uptake but slower turnover of I* and a larger total iodine pool. The thyroids of animals receiving 2 and 3 μg., however, collected less I* but turnover was more rapid and the total iodine pool was reduced. The 4 μg. dose virtually eliminated uptake and secretion of I* from the glands and the total iodine pool indicated accumulation. Under the conditions of this experiment, suppression of iodine turnover was not a direct function of exogenous thyroxine dose. Non-thyroxine iodine is presumably secreted by thyroids of normal and thyroxine-treated chicks.

Comparative effects of hypertonic solutions of sodium succinate, fumarate, acetate, and chloride on renal hemodynamics and PAH extraction in the dog. SAMUEL A. CORSON, ABRAHAM L. SIEGEL,* ELIZABETH J. O'LEARY* AND ROBERT S. PICARD.* *Toledo Hosp. Inst. of Med. Research, Toledo, Ohio.*

Glomerular filtration rate (GFR), renal plasma flow (RPF), and PAH and creatinine extractions (E_{PAH}, E_c) were determined in 11 anesthetized dogs (6 of which were litter mates) by measuring the clearances of creatinine and PAH (C_c, C_{PAH}) and by unilateral renal vein catheterization and arterial blood samples. All solutions were administered at a constant rate of 1.6 ml/mm. Control clearances were determined before and following the use of the experimental solutions by infusing 0.16 M NaCl solutions containing creatinine and PAH. Disodium succinate or fumarate solutions (0.8 and 1.4 M) markedly decreased the E_{PAH}, while equivalent concentrations (1.6 and 2.8 M) of sodium salts of acetate and chloride had no measurable effect. This lack of effect of acetate on E_{PAH} at low PAH plasma concentrations, as contrasted with the significant increase in the Tm_{PAH} by acetate reported by Mudge and Taggart, lends support to their suggestion that acetate influences directly the enzymatic transfer process and does not increase the number of active nephrons. Our data also furnish further evidence for the validity of C_{PAH} at low concentrations as a measure of blood flow through active renal tissues. All the salts in most experiments produced a small increase in the GFR, a marked increase in the RPF, and a consequent decrease in the per cent filtered. This would imply that the increase in RPF was produced chiefly by efferent vasodilatation. The ratio of the RPF derived on the basis of C_{PAH}/E_{PAH} to the RPF derived from C_c/E_c was fairly close to unity (1.1–0.82), in contrast to the marked discrepancies reported for the human kidney by other authors in regard to the clearances of mannitol, endogenous creatinine, thiosulfate and inulin.

Effects of atropine in men walking in moderate heat. F. N. CRAIG. *Med. Div., Army Chemical Center, Md.*

Preliminary results have been obtained on clothed men under various combinations of the following conditions: *C*, 21°C. and 60% relative humidity; *H*, 31°C. and 80% relative humidity; *R*, seated on a stool; *W*, walking at 3 mph on a level treadmill; and *A*, atropine sulfate, 2 mg. in 1-ml. solution injected intramuscularly. Sweat production was measured by weight loss over a 2-hour period. Heart rate was followed continuously with a cardiotachometer. Conditions *CR*, *CW*, *HR* and *HW* provided a range of weight loss

of from 58–1120 gm. The weight losses in conditions *CRA*, *CWA*, *HRA*, and *HWA* were less than the control by amounts ranging from 13–604 grams. The decreases amounted to from 15–54% of the control. The effect of the atropine appeared to be an inhibition of a fraction of the sweat production rather than a definite amount of sweat. Conditions *CR*, *CW*, *HR* and *HW* provided a range of heart rate from 67–121 beats/minute. The heart rate in conditions *CRA*, *CWA*, *HRA*, and *HWA* was from 29–45 beats faster than the control; the increases amounted to from 27–63% of the control. The results appear to favor slightly the view that the increase in heart rate due to atropine is a definite number of beats rather than a fraction of the control rate. Increases in body temperature, when found, did not occur until the tachycardia was fully developed.

Effect of sodium deficient perfusates on the cardiac injury potential. P. F. CRANEFIELD,* J. A. E. EYSTER AND W. E. GILSON.* *Dept. of Physiology, Univ. of Wisconsin, Madison.*

Eyster observed during cardiac activity an injured area becomes electrically positive with respect to the potential of resting uninjured cardiac muscle. This result and results of others showing the action potential in the giant axon and striate muscle exceeding the resting potential are difficult to explain by the Bernstein membrane theory. Hodgkin has shown the amount by which the action potential exceeds the resting potential is determined by the concentration of Na^+ in the surrounding fluid. In testing the effect of Na^+ deficient perfusates on the positive phase of the cardiac injury potential, injury potentials were produced with the Wiggers suction electrode. The heart of Chelydra serpentina was exposed and perfused *in situ* with normal Ringer's and Ringer's in which the NaCl was reduced to one-third of normal, made isoosmotic with glucose. The area of the normal negative phase in millivolt-seconds was 30.67 ± 2.49, the area of the negative phase with sodium deficient perfusates was 29.25 ± 3.63, not significantly different. The area of the normal positive phase was 10.41 ± 1.37, the area of the positive phase with sodium deficient perfusates was 5.79 ± 1.28, significantly different. This decrease in area, the result of a decrease in both amplitude and duration of the positive phase, suggests the mechanism by which injured cardiac muscle becomes electrically positive to resting uninjured muscle is similar to the phenomena explained by Hodgkin on the basis of Na^+ concentration. (Supported in part by the Wisconsin Alumni Research Foundation.)

Oxygen consumption of the rat during partial inanition. CHARLES W. CRAVEN (introduced by H. I. CHINN). *Dept. of Pharmacology and Biochemistry, USAF School of Aviation Medicine, Randolph Field, Tex.*

It has been demonstrated that the resistance to altitude afforded rats on an exclusive carrot diet can probably be attributed to a lowered caloric intake (CRAVEN *et al.*, *J. Aviation Med.* 21: 356, 1950). An attempt has been made to determine whether the partial inanition resulting from this diet also decreases the oxygen consumption of the rat and thus prolongs its survival at altitude. That such a decrease occurs is the basis of this report. Young male rats (age 3–4 months) were maintained on a normal diet of Purina dog chow, raw carrots and tap water, *ad libitum*, at room temperature. After the rats had become acclimated to their environment, normal oxygen consumption levels were determined for each animal. The rats were in the post-absorptive state (no food for 18 hours). A closed-circuit-type instrument was used and only oxygen consumption was measured. Activity of the rat was automatically recorded and the temperature within the chamber was maintained at 27° ± 0.1°C. After normal values were established, each rat was allowed a short rest period and then given an exclusive diet of raw carrots and tap water *ad libitum* for 10 days. Oxygen consumption was determined on the third day of this diet and every 48 hours thereafter. At the end of the 10-day period of partial inanition the rats had lost 15% in body weight and the oxygen consumption had decreased 20% below the normal level.

Nerve sheath as a barrier to the action of certain substances. FREDERICK CRESCITELLI. *Dept. of Zoology, Univ. of California, Los Angeles.*

A technique has been developed with which the external connective tissue sheath (epineurium) of the bullfrog sciatic-peroneal nerve may be removed from the main portion of the nerve and then replaced. Using this technique it has been possible to show that the action of certain compounds which block nerve impulses and which depolarize the nerve membranes is not quantitatively the same on resheathed nerves as on desheathed nerves. The results indicate that with respect to these substances (D-amidone, L-amidone, D,L-amidone, benadryl, histadyl and thephorin) the resheathed nerves behave somewhat like the normal nerves and in some cases exactly like the normal nerves. Both the resheathed nerves and the normal nerves show, in comparison with the desheathed preparations, a slower onset of action and a slower rate of action in response to these drugs. In addition, unsheathed nerve trunks, produced by slipping off the epineurium from previously resheathed preparations, act more like desheathed rather than resheathed nerves. These findings are in agreement with the results of previ-

ous experiments which suggest that the sheath is a barrier to the action of a number of compounds. They are difficult to interpret in terms of the view that alterations in properties of nerve fibers produced by desheathing explain the much more rapid action of substances on nerves whose sheaths have been removed.

Effects of Protoveratrine on cerebral hemodynamics and oxygen metabolism in hypertensive subjects. CHARLES W. CRUMPTON,* WALTER C. KLINGENSMITH,* NELLY J. KEFFER* AND JOSEPH H. HAFKENSCHIEL. *Robinette Fndn., Med. Clinic, Hosp. of Univ. of Pennsylvania and Dept. of Pharmacology, Med. School, Univ. of Pennsylvania, Philadelphia.*

Cerebral blood flow and metabolism were studied in uncomplicated hypertensive patients before and one hour after parenteral Protoveratrine. Since the route of administration presented a problem in evaluating the response to the drug both the intramuscular (7 patients, 6–8 μg/kg.) and intravenous (7 patients, 2 μg/kg.) routes were investigated. Following intramuscular injection mean arterial pressure fell gradually during the first hour and remained at a low level (109 mm. Hg) for approximately 3 hours. Intravenous dosage reduced the pressure promptly, the lowest level being observed at 15 minutes (86 mm. Hg). A gradual increase followed, and the pressure stabilized in one hour at a level significantly lower than before injection (112 mm. Hg), at which time the second measurements were made. Cerebral vascular resistance was significantly reduced in both groups ($P < .01$). Cerebral blood flow decreased from 61–49 cc/100 gm/min. ($P < .05$) in the intramuscular series, but there was no essential change following intravenous administration. Cerebral oxygen consumption did not change in either group. Arterio-venous oxygen difference increased 1.1 vol.% after intramuscular Protoveratrine and 0.6 vol.% following the intravenous route. However, these changes were not statistically significant. Although the degree of reduction in mean arterial blood pressure was comparable in both groups (30% and 25%), the mean cerebral blood flow decreased 20% in the intramuscular series and only 6% in the intravenous group. These data indicate a significant relaxation of cerebral vascular tone during the hypotensive period following intravenous Protoveratrine and favor the employment of this route in performing circulatory studies.

Disappearance rate of exogenous thyrotrophin from blood of normal and hypophysectomized rats. SAVINO A. D'ANGELO. *Dept. of Anatomy, Jefferson Med. College, Philadelphia, Pa.*

Blood levels of thyroid stimulating hormone (TSH) in adult male rats (250–325 gm.) and hypophysectomized partners (150–170 gm., 3–7 weeks post-operative) were determined (stasis tadpole technique—D'ANGELO AND GORDON. *Endocrinology* 46: 39, 1950) at various time intervals (6 mins., 1 hr., 5 hrs., 24 hrs., 48 hrs.) after a single intracardial injection of previously standardized purified hormone. TSH recovery values in sera indicated that the disappearance rate of exogenous hormone from the blood was greater in intact than in hypophysectomized animals. The serum concentration of TSH in intact rats fell logarithmically against time, with approximately 30% of the theoretically expected concentration recoverable at the 6-minute interval, but with less than 3% and 1% detectable at 1 and 5 hours respectively. Extrapolated values were such as to suggest almost complete recovery of the hormone at the zero time interval. In hypophysectomized animals, blood TSH levels were approximately 10-fold greater at corresponding time intervals (1 and 5 hrs.). Hormone could be detected in the circulating blood of hypophysectomized rats 24 hours after injection, but had vanished by 46–48 hours. Interpretation of the differences between the normal and hypophysectomized rat as regards blood disappearance rate and metabolic fate of TSH is made. (Supported, in part, by a grant (RG-2407) from the Public Health Service.)

Acclimatization to cold in man. FARRINGTON DANIELS, JR., DAVID C. FAINER, C. LAWRENCE BOMMARITO AND DAVID E. BASS (introduced by H. S. BELDING). *Army Med. Dept. Research and Graduate School, Washington, D. C., and Quartermaster Climatic Research Lab., Lawrence, Mass.*

During the summer, 12 men were exposed to a temperature of 60°F. for 12 days, and after a 10-day interval for another 5 days. The men were in the cold room for 19¾ hours out of each day, the exposure being interrupted for meals and for a baseline period each morning in which the men lay quietly at 85°F. dressed only in shorts. Alterations in response to the abrupt change from 85° to 60° furnish evidence of acclimatization. At 60°F., many of the responses of the body to cold exposure are elicited but damage due to cold is not a problem. Field reports of cold acclimatization emphasize the apparent role of small coordinated changes in many responses rather than striking changes in any one function. The results of this study, in which a number of physiological responses were measured, agree with this concept. Indications of reduced physiological strain with repeated application of the same cold stress were most evident in changed distribution of body heat. There was lessened decrement in rectal temperature between the beginning of acute exposure and 2 hours of exposure during the course of acclimati-

zation. For given cold exposures, forehead temperatures became higher and hand temperatures lower. Subjective improvement in cold tolerance was present in 11 men, in most on the fourth or fifth day. No changes in basal metabolic rate were found.

Acid secretion and acetoacetate utilization in mouse stomachs in vitro. HORACE W. DAVENPORT AND VIRGINIA J. CHAVRE.* *Dept. of Physiology, Univ. of Utah College of Medicine, Salt Lake City.*

Acid secretion and acetoacetate utilization were studied in mouse stomachs in vitro using acetoacetate concentrations from 0–12mM/liter. In the presence of 0.1 mg.% carbaminoylcholine both acid secretion and acetoacetate disappearance are linear functions of acetoacetate concentration. If no correction for changes in pH of the buffer resulting from acetoacetate disappearance is applied, 1.18 moles of acid are secreted for each mole of acetoacetate used. In the absence of carbaminoylcholine, acid secretion is entirely independent of the acetoacetate concentration, and it is equal to that occurring when no exogenous substrate is present. However, the disappearance of acetoacetate in the absence of carbaminoylcholine is a function of the acetoacetate concentration, and at an acetoacetate concentration of 12mM/liter the disappearance of acetoacetate is about 70% that occurring in the presence of carbaminoylcholine. Thiocyanate at 30 mg.% strongly inhibits acid secretion in the presence or absence of carbaminoylcholine, and in both circumstances the disappearance of acetoacetate is the same as that occurring in the absence of thiocyanate and carbaminoylcholine.

Studies of renal function and metabolic balances in dogs with experimental pericarditis. JAMES O. DAVIS, ALAN E. LINDSAY AND JAMES L. SOUTHWORTH (introduced by ROBERT W. BERLINER). *Section on Kidney and Electrolyte Metabolism, Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and U. S. Marine Hospital, Baltimore, Md.*

An experimental preparation for study of the pathogenesis of edema was produced by implantation of cellophane into the pericardial cavity. Changes in Cr and PAH clearances, Na, K, Cl, N, and water balances, plasma electrolytes, T-1824 dye space, hemoglobin, hematocrit, plasma proteins, body weight, arterial and right ventricular pressure, and central and peripheral venous pressure were studied for a 3- to 4-week control interval and during the experimental period. The experimental period was represented by two phases of fluid retention. The acute phase was characterized by edema and lasted 10–12 days. In the chronic phase ascites was the principal depot for electrolyte and water storage; this phase lasted as long as 6 months. These preparations displayed markedly positive Na and Cl balances and increased T-1824 dye space, body weight, and venous pressure during both phases. C_{Cr} and C_{PAH} were elevated during the acute phase but FF was normal or slightly reduced. During the chronic phase renal function remained elevated or returned to the control level unless protein depletion supervened; in this event C_{Cr} and C_{PAH} fell below the control level and FF increased. Available data have yielded inconclusive evidence concerning the effect of venous hypertension on renal tubular reabsorption of electrolytes and water.

Effects of potassium cyanide on growth and respiration in tetrahymena *geleii* furgason. RICHARD E. DEAN (introduced by CHARLES G. WILBER). *Univ. of Nebraska, Lincoln.*

The effects of potassium cyanide on growth and respiration in tetrahymena *geleii* were ascertained for concentrations ranging from 10^{-3}M to 10^{-12}M. Concentrations of 10^{-3}M resulted in cytolysis of the organisms within a few hours after introduction of the cyanide into the media. In 10^{-4}M KCN growth was inhibited by 18% and respiration was inhibited by 57.3%. Solutions of 10^{-8}M KCN produced a 63% acceleration in growth over the controls but was not accompanied by an increase in respiratory rate. An increase in respiration of 7.3% over the controls was noted in solutions of 10^{-9}M concentration but was not considered to be significant. No differences between the sizes attained by the cells subjected to cyanide and the controls were noted. Microscopic examination of the organisms showed that potassium cyanide increased transparency of the cells and resulted in a great reduction in the number of vacuoles (20–50 reduced to 3–5). Movement of the organisms was slowed and they collected near the surface of the media. The time required for these effects to become apparent and the degree to which they were observed are dependent upon the cyanide concentration. At extremely low concentrations (10^{-11}M and 10^{-12}M) these effects were not observed.

Growth hormone and water metabolism in hypophysectomized dog. R. C. DE BODO, I. L. SCHWARTZ,* J. GREENBERG,* M. KURTZ,* D. P. EARLE, JR. AND S. J. FARBER.* *Depts. of Pharmacology, Physiology and Medicine, New York Univ. College of Medicine, New York City.*

Water metabolism in the hypophysectomized dog is disturbed. Only a small fraction of an orally administered water load (40 ml/kg.) is excreted within 3 hours in contrast to the quantitative excretion seen in normal dogs (*Federation Proc.* 9: 30, 1950, and SCHWARTZ *et al.* this issue). This

disturbance does not reveal itself in the daily water exchange. Following hypophysectomy dogs develop a transient severe diabetes insipidus. Within a month the water exchange returns either to pre-operative levels or stabilizes at mildly polyuric ones. Growth hormone (Armour), 1 mg/kg. daily, given intramuscularly for 14 to 33 days progressively increased (sometimes to normal) the response to water loads in every dog. The daily water exchange progressively rose reaching levels seen in surgically induced, severe diabetes insipidus. The markedly impaired glomerular filtration rate and renal plasma flow seen in the hypophysectomized dog also increased during growth hormone administration, but remained far below pre-operative values. The increased glomerular filtration rate appears to play a role in the increased excretion of water loads and the increased water exchange. A direct hormonal effect on the renal tubular reabsorption of water cannot be excluded. Adrenocorticotrophic and thyrotrophic hormones present in the growth hormone preparation were not responsible for the changes noted above. It is suggested that growth hormone is one of the factors concerned with the regulation of water metabolism.

Motor representation within sulci of the cat's frontal lobe as studied by chronic implanted electrodes. JOSE M. R. DELGADO (introduced by ROBERT B. LIVINGSTON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

From 2–4 groups of 7 electrodes each are stereotaxically implanted with aseptic precautions into the frontal lobe of cats in order to activate cortex which is buried within the sulci. The electrodes are fixed to the skull and extend beneath the skin to a collar where they are connected to a miniature radio tube socket. Controlled rectangular pulses are delivered to the socket by attaching during the experiments a long lead and socket plug. The cats are tested without anesthesia and with practically complete freedom of movement. The contralateral hind extremity is represented in the anterior two-thirds of the sulcus cruciatus. The anterior half of the sulcus coronalis is concerned with motility of the contralateral forelimb. The sulcus rhinalis anterior has systematic motor representation of the anterior half of the body, the forelimbs being represented in the dorsal and the face in the ventral portions of the sulcus. In general, responses are contralateral, occasionally bilateral and sometimes ipsilateral. From a zone close to the facial representation respiratory responses are elicitable. Stimulation of this hidden cortex causes no apparent emotional disturbance of the animals. Often the evoked movements are directed purposefully by the conscious animal. Prolonged repeated excitation of the same point may be followed by 'spontaneous' periodic recurrences of the response during 2 or 3 hours following cessation of stimulation. Responses to stimulation of the hidden motor cortex will summate with spontaneous movements resulting in dysmetria.

Neuromuscular organization of single motor units. J. S. DENSLOW AND OLWEN GUTENSOHN.* *Kirksville College of Osteopathy and Surgery, Kirksville, Mo.*

Single motor units in the peroneus longus muscle (cat) have been activated reflexly or by electrical stimulation of a bundle of anterior root fibers which contains a single fiber to the peroneus longus. Observations previously reported (*Federation Proc.* 9: 1, 1950) have been modified and extended. With improved stimulating technics, it is apparent that single units are smaller than previous observations indicated. In these experiments, some employing reflex activation of the unit and some electrical stimulation, the following have been observed; *a*) every unit appeared to have fibers in the middle one-third of the muscle and in either the proximal or distal one-third, but not in both, and in both the interior and surface of the muscle; *b*) the action potential in different parts of the muscle was synchronized but not simultaneous, the minimum and maximum interval between the beginning of the earliest action potential and the end of the last being 6.4 and 8.8 msec., respectively; *c*) the main spike occurred at different times in different locations; the occasional finding of 2 large negative spikes within the pick-up range of one pair of electrodes indicates the presence of 2 or more bundles of fibers which, although quite close together geographically, contribute to the temporal dispersion in the contraction of the unit; *d*) there is definite evidence that a certain degree of concentration of fibers in the unit does exist, and *e*) that such concentrations are not always limited to one narrow strip in the long axis of the muscle.

Effect of *p*-aminohippurate on renal tubular reabsorption of urea in normal man. RAYMOND J. DERN AND THEODORE N. PULLMAN (introduced by ALF S. ALVING). *Univ. of Chicago, Dept. of Medicine, Chicago, Ill.*

The fraction of filtered urea undergoing tubular reabsorption in 12 normal men was measured before and after infusion of *p*-aminohippurate in amounts sufficient to saturate the tubular excretory mechanism. Each reabsorptive fraction was the average obtained on 2–4 clearance periods. Urine flows well above the 'augmentation limit' were obtained by water diuresis. When the subjects were consuming a high protein ration (2–3 gm/kg/day), the urea reabsorptive percentage exhibited an average fall of 3.12. On a medium

protein intake (1 gm/kg/day), the average depression was 0.85. On a low protein diet (0.3 gm/kg/day), the urea reabsorptive percentage showed a slight rise (0.54) after *p*-aminohippurate infusion. The only change that could be adjudged statistically significant was the depression on the high protein regimen ($P = 0.1 - 0.02$ by the t test of Fisher). The observed depression of urea reabsorption was independent of changes in water reabsorption since the latter was greater after PAH loading than it had been during the control periods.

Limiting mechanism in tarsal chemoreception. V. G. DETHIER. *Dept. of Biology, Johns Hopkins Univ., Baltimore, Md.*

The comparative effectivensss of the first 8 normal alcohols in stimulating the tarsal chemoreceptors of blowflies has been tested using 3 solvents: water, ethylene glycol, and mineral oil. Alcohols in aqueous solution stimulate at logarithmically decreasing molar concentrations as chain length increases. There is a sharp break at pentanol in the curve which describes this relationship. It has no counterpart in any of the tabulated chemical or physical properties. In mineral oil solution, alcohols from butanol to pentanol are approximately equally stimulating; those from hexanol to octanol, equally so at lower concentrations. However, a decrease in effective concentration from butanol to octanol is apparent. The line describing this relationship intersects the water line near pentanol. The glycol line is intermediate in position. When thresholds of alcohols in aqueous solution are expressed in thermodynamic activities, they bear the same relationship to one another as oil values expressed in mol fractions do to each other. Thus the first 5 alcohols stimulate at progressively increasing activities; the last 4, at progressively decreasing activities. The persistence of a discontinuity near pentanol indicates that this chemoreceptive system either does not conform to the rule, which has such widespread application to narcosis phenomena that equal effects are produced at equal activities, or that the limiting mechanism being measured obscures the underlying relationship. Data support the hypothesis that the limiting mechanism involves a two-phase system of such a nature that smaller molecules gain access to receptors through an aqueous phase while larger aliphatic molecules penetrate through or accumulate in a lipoid phase.

Anaphylaxis in the rat (motion picture). PETER B. DEWS AND CHARLES F. CODE. *Section on Physiology, Mayo Fndn., Univ. of Minnesota, and Mayo Clinic, Rochester, Minn.*

The film contrasts the effects of intravenous injections of 0.8–1.0 ml. of horse serum into normal and adrenalectomized rats, all of which had been given subcutaneous injections of horse serum 14–24 days previously. The minimal reactions of intact animals are illustrated. In nearly all of the adrenalectomized animals a characteristic anaphylactic reaction developed. A number of rats displaying the sequence of this reaction are shown. The most consistent signs seen during the reactions were difficulty in breathing and weakness. A third sign seen only in animals which had been adrenalectomized prior to administration of the sensitizing injections was a spasm complex in which the animal rubbed its nose in its flank and, rolling sideways, clasped the lower part of its abdomen with its hind legs. The spasm complex often culminated in convulsive movements of the animal's entire body. The episodes usually lasted only a minute or two, but were often repeated. The motion picture also illustrates the scoring system which has been devised to allow rough estimation of the severity of the anaphylactic reaction. Examples are shown of its application to studies of the changes produced in the reaction by variations in the time of adrenalectomy with respect to the time of sensitization and by administration of cortisone and desoxycorticosterone acetate.

Observations on oxygen consumption of rat kidney slices in relation to conditions affecting renal water reabsorption. INGRITH J. DEYRUP. *Depts. of Zoology of Barnard College and Columbia Univ., New York City.*

Mammalian kidney tissue *in vitro* shows a relative increase in oxygen uptake when treated with crude extracts of the posterior lobe of the pituitary gland (CUTTING AND MCCANCE. *J. Physiol.* 104: 288, 1945–46). Using the Warburg technique, it has been found that the respiration of rat kidney slices is consistently elevated, as compared with controls, in the presence of 0.2–1.0 U of Pitressin (a commercial preparation of the pressor and antidiuretic principles of the posterior pituitary). The effects upon kidney metabolism appear to vary with the pH of the medium, the presence or absence of substrate, and the water load of the animal from which the renal tissue is obtained. The most marked effect was noted in 7 experiments carried out at low pH (4.6–6.9) without substrate, where respiration averaged 38% higher in the presence of Pitressin than in control tests. Oxygen uptake of kidney slices obtained from animals sacrificed during water diuresis was not increased by Pitressin, whereas in 7 out of 8 experiments on animals dehydrated by 24–73 hours of water deprivation, the respiration of Pitressin-treated slices was elevated consistently. Preliminary findings suggest that the effect of Pitressin on liver slices does not parallel the action on kidney tissue. A possible relation between the effect of Pitressin on renal

respiration *in vitro* and the increased water reabsorption by kidney tubules following the administration of posterior pituitary antidiuretic principle will be discussed.

Relation of EMF to permeability of frog skin to phosphorous. H. W. DISERENS (introduced by C. E. HALL). *Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.*

It has been suggested that electrical phenomena are important in regulating membrane permeability. Availability of material and ease of measurement of the EMF of frog skin make it a satisfactory tissue for exploration of such a general hypothesis. The relation of the inherent EMF to the permeability of frog skin to phosphate ion has been investigated using P^{32} as tracer. Excised skin was placed between glass chambers and frog Ringer's, pH 7.0, added to both chambers. Potential levels were recorded automatically at one-minute intervals. Once the EMF had become constant, P^{32} (as NaH_2PO_4) was added to the solution on the morphological outside of the skin or to the solution inside, activity 100,000 cts/ml/min. Samples from the opposite chamber were taken at 30-minute intervals during a 2-hour period and activity determined. The data indicate, under the conditions studied, that skin displaying a relatively constant EMF (±17% of equilibrium) is impermeable to phosphate ion when the ion is placed outside the membrane. This impermeability (relative to transfer through the skin) appears without regard to absolute potential differences. With the ion inside, only one of five skins exhibited a low but significant permeability to this ion, activity in the opposite chamber at 120 minutes, 55.7 ± 0.81 cts/ml/min. Counts of the skin exposed to the tracer shows a remarkable binding of the ion within the membrane, the amount of P^{32} bound some four times greater when the tracer is added inside.

Reciprocal inhibition of spinal motoneurons. A. S. DONTAS,* D. C. PETERS* AND ROBERT GESELL. *Physiology Lab., Univ. of Michigan, Ann Arbor.*

Activity of phrenic neurons was studied by recording electric potentials of emergent fibers with d. c. amplifier. Inhibition of inspiration produced by stimulation of predominantly expiratory excitatory nerves, superior laryngeal or vagus, e.g., resulted in depression or elimination of slowly oscillating negative potentials and decrease or arrest of action potentials. The end of stimulation was followed by gradual return of electrotonic potential to original level and to normal oscillation together with return of action potentials and rhythmic breathing. Excitation of these nerves is supposed to stimulate both half-centers of respiration though unequally, so that central reciprocal inhibition of inspiration occurs indirectly through activation of its antagonist. This was tested by stimulation of superior laryngeal under extreme contrasting conditions, i.e. during supernormal inspiratory and supernormal expiratory activity against appropriate closure of trachea. Stimulation of superior laryngeal nerve during supernormal inspiratory activity potentiates that activity still more and delays the expiratory phase; whereas stimulation during supernormal expiratory activity increases prevailing inhibition of inspiratory neuron. Observed electrical potentials conform with above. Stimulation of predominantly inspiratory nerves giving normally powerful phrenic discharges is, on the other hand, able to increase existing inhibition of the same neurons if delivered when the antagonists are superactive. *Thus prevailing state of activity of half-centers* rather than particular fibers stimulated determines the type of response to reflex stimulation, a fact supporting the concept of reciprocal interlocking of antagonistic half-centers, and avoiding the need of invoking 'positive' potentials on nerve cell bodies in explanation of inhibition.

Measurement of secretin content of intestine in hypophysectomized and normal rats. J. E. C. DORCHESTER* AND R. E. HAIST. *Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada.*

Using the Latin Square experimental design, it was shown that a basic straight line relationship existed between log dose of secretin and the response of pancreatic secretion in rabbits. This relationship and experimental design were then made the basis of a method of secretin assay. This method of assay was used in testing the secretin content of extracts of the entire small intestine of rats. It was found that hypophysectomy led to a reduction in the secretin per gram of dried intestine and also to a decrease in the total content of secretin in the small intestine of the rat. It would appear that the pituitary gland exerts a definite influence on those endocrine structures of the intestine concerned with the formation and liberation of secretin, though the mechanism of this action has not been established as yet.

Topical antiphlogistic action of cortisone. THOMAS F. DOUGHERTY. *Dept. of Anatomy, Univ. of Utah, Salt Lake City.*

Cortisone is antiphlogistic when given systemically (DOUGHERTY, T. F. AND G. L. SCHNEEBELI. *Proc. Soc. Biol. & Med.* in press). To test whether cortisone has a local antiphlogistic action, it was injected into loose connective tissue of adrenalectomized CBA mice simultaneously with a phlogogenous agent. Histamine diphosphate (0.2 mg. in 0.25 cc. saline) injected subcutaneously was the standard inflaming stimulus. The degree of inflammation was measured at 6, 12 and 24 hours by counting the number of damaged fibro-

blasts and non-autochthonous cells per mm.^{3} of air-dried May-Grünwald-Giemsa stained spreads of the inflamed tissue. Graded amounts of cortisone acetate from 0.25 to 50 μg. added to the histamine solution inhibited the inflammation produced by histamine alone. A smaller dose of cortisone (0.125 μg.) had no inhibitory action. When the amount of histamine was increased, larger amounts of cortisone were required to inhibit inflammation. Circulating eosinophils per mm.^{3} tail vein blood were determined in all experiments to ascertain whether the cortisone doses employed had systemic effects. Doses between 5 and 20 μg. cortisone produced eosinopenia when injected alone but not when given with the standard inflaming dose of histamine. Thus, the local antiphlogistic effect of cortisone is not produced by way of a systemic mechanism. The findings indicate that the local 'utilization' of the hormone is increased in an inflamed area in proportion to the intensity of the inflammatory process. (Supported by a grant from the Life Insurance Med. Research Fund.)

Spectrophotometric studies of the dye-albumin complexes. PHILIP DOW. *Dept. of Physiology, Med. College of Georgia, Augusta.*

This report completes a protracted series of studies (*Federation Proc.* 1945 and 1947; *Am. J. Physiol.* 1950) comparing the biological and physico-chemical behavior of the two most nearly intravascular dyes, Brilliant Vital Red and Evans Blue (T-1824). The complexes of these dyes with serum albumin (gift of Armour Laboratories) at different molar ratios are examined spectrophotometrically, and interconversion tendencies are analyzed. As before, emphasis is on practically salt-free solutions, with separate consideration of the effect of NaCl on the curves. The results are in harmony with the previously expressed hypothesis that an important factor is the dispersion stability or degree of local aggregation of the dye molecules.

Effect of hypoxia on pulmonary vascular pressures in man. JOSEPH T. DOYLE,* JOSEPH S. WILSON* AND JAMES V. WARREN. *Emory Univ. School of Medicine, Atlanta, Ga.*

Sizable variations in pulmonary vascular pressures can be induced in normal humans by expansion or reduction of effective blood volume. Pulmonary arterial and capillary pressures increase or decrease proportionally to blood volume, without alteration in pulmonary pressure gradient. On the other hand, patients with certain circulatory abnormalities may show an abnormally high pulmonary pressure gradient despite normal or small blood volumes. These observations suggest pulmonary vascoconstriction. As an approach to the exploration of this possibility, 14 normal individuals and 10 patients with cardiopulmonary abnormalities were subjected to inhalation of 10% oxygen for short periods. Cardiac output was determined by direct Fick and dye dilution techniques. The pulmonary arterial pressure (average mean 12 mm. Hg) of all normal subjects rose an average of 8 mm. Hg during hypoxia. Pulmonary capillary pressure was unchanged so that the pulmonary pressure gradient was increased. Pulse rate increased moderately. Blood flow usually increased, but not proportionally to the degree of pulmonary hypertension. Systemic vascular resistance tended to fall. 'Pulmonary blood volume' was questionably increased. In the abnormal group, hypoxia produced less constant alterations and occasionally no change in pulmonary arterial pressure. In these latter subjects, there was evidence of markedly expanded blood volume.

Response of *Chilomonas paramecium* to ionizing radiation. HOWARD S. DUCOFF* AND JOHN O. HUTCHENS. *Argonne Natl. Lab., Chicago, and Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

X-ray doses of less than 10,000 r (air) have no apparent effect on the rate of growth or cell division in cultures of *Chilomonas*, while 90,000 r result in death of about 40% of the cells and a block to cell division that is irreversible within 30 hours. At intermediate doses some cell deaths occur, and there is a cell division block, the duration of which appears to be a direct function of dose. Growth then resumes at the control (logarithmic) rate. During the period of block, the cells increase greatly in volume, as do the starch granules. Just prior to resumption of cell division after being blocked by nitrogen mustard (HN-2), the cells are somewhat more radioresistant. Such cells are resistant to additional exposure to HN-2 (HUTCHENS AND PODOLSKY. *Federation Proc.* 9: 65, 1950). This effect is not due simply to a difference in radiosensitivity between dividing and 'resting' cells, since cells prevented from growing and dividing by incubation in nitrogen free media are no more resistant than are growing cells. Such cells transferred to complete medium at various intervals after irradiation exhibit the delay expected at that dose, plus any delay exhibited by unirradiated controls. Thus, recovery from x-radiation as from nitrogen mustard damage appears to require not a fixed time but a fixed amount of protoplasmic synthesis, probably of nitrogenous materials.

Effect of large quantities of parenteral sodium chloride and fluid on plasma potassium and intracellular potassium balance. ABRAHAM DURY. *Dorn Lab. Med. Research, Bradford Hospital, Bradford, Pa.*

Parenteral electrolyte replacement in surgical

patients until recently had been entirely dependent upon sodium and chloride. Recognition recently that potassium deficits are incurred in these patients which may lead to serious clinical and biochemical alteration has reopened the problem of electrolyte replacement. Sodium and potassium balance and serum sodium, potassium and bicarbonate levels were studied in 3 groups of patients after gastrectomy during the time they were given parenteral fluids only. *Group I* was given large quantities of fluid and large amounts of sodium chloride replacement; *group II* was given moderate to large quantities of fluid and very low amounts of sodium chloride; *group III* was given adequate fluid replacement, but low sodium chloride in conjunction with potassium phosphate as replacement electrolytes during the first few days after surgery. Data will be presented showing that hypokalemic levels in *group I* was associated with large negative balance of intracellular potassium. In *group II* the plasma potassium levels remained fairly normal as a result of contraction of the extracellular space but this group too had a large negative intracellular potassium balance. The result in *group III* was most favorable with very small or no changes in plasma potassium level, extracellular fluid space and intracellular potassium balance. These results in relation to the excellent post-surgical recovery will be discussed.

Effects of nitroglycerine on myocardial oxygen consumption in dog. R. W. ECKSTEIN, WILLIAM NEWBERRY AND J. MCEACHEN. *Dept. of Medicine, Western Reserve Univ., Cleveland, Ohio.*

These experiments were designed to test the hypothesis that nitroglycerine antagonizes the myocardial anoxating properties of epinephrine and similar agents by reducing myocardial oxygen consumption. Total left coronary flow was measured with a rotometer. A coronary sinus cannula permitted venous sampling for A-V oxygen differences and in some cases continuous venous oxygen changes with a Waters Conley oximeter. Preliminary experiments showed that the intracoronary injection of nitroglycerine produced large increases in coronary flow and coronary sinus oxygen content without changes in oxygen consumption. The intracoronary injection of epinephrine produced the usual large increases in flow and oxygen consumption. The intracoronary injection of nitroglycerine at the peak of the epinephrine response produced a further increase in coronary flow, a decrease in the A-V oxygen difference but no significant change in myocardial oxygen consumption. In a third series of experiments studies were made on the effect of nitroglycerine on the changes produced by stimulation of the cardiac accelerator nerves. Such stimulation produced flow increases of 175% and increases in oxygen consumption of about 200%. When nitroglycerine was injected at the peak of the nerve stimulation response there was a further increase in flow without a significant change in oxygen consumption. Therefore in these experiments physiologically active amounts of nitroglycerine do not modify myocardial oxygen consumption either before or after the administration of epinephrine or stimulation of the cardiac accelerator nerves.

Survival of rats adrenalectomized after x-irradiation. ABRAHAM EDELMANN AND SEYMOUR KATSH.* *Biology Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Rats were adrenalectomized at various times after a dose of whole body X-irradiation (600 r, 160 kvp., 10 ma., 0.2 mm. Cu, a dose which does not kill normal control rats in this laboratory), and the time of death noted. It was observed that the life span was reduced when the time interval between irradiation and adrenalectomy was decreased. This relation was not linear, a greater effect being noticed at the shorter intervals. If the irradiation was given 5 or more days before the adrenalectomy, the life span of the irradiated-adrenalectomized rats approached that of the unirradiated-adrenalectomized rats. This may be interpreted to mean that the need of the animal for adrenal hormones to survive is most acute immediately after irradiation and rapidly diminishes after 3–5 days. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Blood flows in the hyperthermic dog following cord section and lumbar sympathectomy. H. E. EDERSTROM. *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Blood flow distribution in anesthetized dogs with spinal cord transection at *T6* was studied with the photoelectric plethysmograph at normal body temperature and during hyperthermia induced by radiant heat. In animals kept at normal body temperature, section of the cord was followed by a fall in blood pressure over a period of hours. Blood flows in the hind foot pad and intestine showed a rise after cord section, followed by a decline. Tongue blood flows showed little change throughout the experiment. In a second experiment body temperature was elevated following cord section and the above observations made at intervals until lethal hyperthermia ensued. Cord section initially produced the effects on blood pressure and flows described above, but on raising body temperature the blood pressure fall was accelerated, and blood flows in the hind foot and intestine fell below control levels. Tongue blood flows were relatively unchanged, whereas in the

intact animal a marked rise in flow accompanied hyperthermia. In a third experiment, hyperthermia was induced in dogs with unilateral lumbar sympathectomy performed several weeks or months previously. Initial observations on the anesthetized animal disclosed blood flows on the intact side to be about double those on the operated side. Elevation of the body temperature to 42°C. was accompanied by a decrease in blood flow in both hind foot pads, which was somewhat more pronounced on the intact side. Tongue blood flows increased markedly with hyperthermia in this group. (Aided by a grant from the U. S. Public Health Service).

Incubation hemolysis of human erythrocytes. M. M. Ellis and M. D. Ellis.* *Dept. of Physiology and Pharmacology, Univ. of Missouri Med. School, Columbia.*

Hemolysis of erythrocytes in 1:100 dilutions of dog blood in buffered salines ranging from 0.41 to 1.28% incubated at 37°, 42° and 45°C. for 22 hours or less varied directly although not proportionately with the osmotic tonicity of the saline (Ellis and Ellis. *Proc. Soc. Exper. Biol. & Med.* 74: 251, 1950). Extending these studies to human bloods it was found that little hemolysis was obtained by incubation unless washed corpuscles were carried in markedly hypertonic salines at the higher temperatures. Bloods from 28 university students, apparently in good health, were drawn by venopuncture and the erythrocytes washed with buffered saline. The final suspensions contained 1:100 corpuscles and 1:65,000 serum in terms of whole blood. These suspensions were made up in 1, 2, 2.5, 3 and 4% salines buffered to pH 7.4 and incubated at 45°C. for 5 hours. The mean hemolysis values, considering hemolysis in 1% as 100, were: 1%, 100; 2%, 165; 2.5%, 251; 3%, 216; 4%, 104. Applying Fisher's '*t*' formula to these means the differences between the hemolysis in 1% and 2%, 2% and 2.5%, and 3% and 4% were definitely significant, but the difference between 2.5% and 3% series was not significant, i.e. the hemolysis was progressively greater from 1% saline to a maximum between 2.5% and 3% and distinctly less in 4%. Hemolysis of blood from 18 ambulatory hospital patients followed essentially the same pattern although showing certain groupings with reference to the disease involved.

Growth hormone induced diabetes in the rat. Frank L. Engel, W. J. Coggins,* W. Lynn* and A. Viau.* *Dept. of Medicine, Duke Univ., Durham, N. C.*

Purified anterior pituitary growth hormone has been shown to be diabetogenic in dogs, cats and partially depancreatized or alloxanized rats, but not heretofore in rats with intact pancreas. The present study demonstrates that growth hormone (Armour) may be diabetogenic in tube-fed rats treated with doses of ACTH (Armour) which, by themselves, are not diabetogenic. Five rats, initial weights 250–300 gm., were fed a high carbohydrate diet (Ingle) supplying 4.3–7.5 gm. of available carbohydrate/100 gm. body weight and were treated with hormones intermittently from 35–100 days. Following adaptation to tube feeding, there was no glycosuria, and blood sugar levels one hour after tube-feeding were less than 150 mg.% at all levels of diet. 1.5–8.0 mg. of ACTH daily induced no significant glycosuria and maximal blood sugars of 190 mg.%, except in the rat that received the largest diet. This rat excreted up to 1 gm. of glucose/day with a maximal blood sugar of 296 mg.%. In one rat receiving rapid increments of diet volume, 5 mg. G.H. daily induced 5 gm. glycosuria and a blood sugar of 397 mg.%. Other rats receiving G.H. showed only transient hyperglycemia one hour after feeding, without glycosuria. All rats receiving ACTH and G.H. developed glycosuria up to 7 gm. and maximal hyperglycemia of 596 mg.%. Glycosuria varied with the dose of G.H. and was sustained as well by 3 as by 8 mg. of ACTH. Glycosuria varied with diet volume. It is concluded that G.H. is diabetogenic in the intact rat, the adrenal cortex playing a synergistic role.

Elevation of serum mucoprotein by parathyroid extract. Milton B. Engel (introduced by Hubert R. Catchpole). *Univ. of Illinois College of Dentistry, Chicago.*

There is a relation between the resorption of the glycoprotein containing ground substance of bone and cartilage and the blood mucoprotein level following the administration of small and large doses of parathyroid extract. Young rats receiving 10–1600 U of parathormone were killed at 1–96 hours. Blood was withdrawn for mucoprotein determinations. Thin pieces of knee-joints and kidney were fixed by freezing-drying for histochemical and morphological studies. Microscopic evidence of accelerated cartilage and bone resorption was present in animals receiving over 500 U. Some bone spicules stained more intensely; others were reduced to fibrillar fragments. Macrophages in the primary spongiosa contained a greater number of glycoprotein granules and insoluble phosphate and/or carbonate. These changes are interpreted as evidence of breakdown of ground substance. Normal serum mucoprotein levels, expressed in terms of a mannose-galactose standard, and tyrosine, were: carbohydrate 15.9 ± .8 (S.E. of mean) mg/100 cc., tyrosine 4.1 ± .2 mg/100 cc. Eighteen hours after 10–30 U of parathormone the values were elevated to around 25 and 6, respectively. Massive doses (800–1000 U in

72 hours) produced values as high as 73 and 21, respectively. Intermediate dosage led to intermediate mucoprotein levels. Anuria and gross kidney changes developed in rats receiving over 1000 U. In sections, large aggregates of glycoprotein granules were observed in the tubule cells. The lumina of many tubules held granular or homogeneous carbohydrate-containing material, which was sometimes associated with insoluble phosphates. This carbohydrate-containing material is thought to be derived from the serum mucoprotein.

Further observations on certain responses to massive doses of epinephrine. HIRAM E. ESSEX. *Mayo Fndn., Rochester, Minn.*

Vigran and Essex (*Am. J. Physiol.* 162: 230, 1950) have reported that a tolerance to epinephrine greater than three times the surely lethal dose can be acquired by dogs. The mechanism responsible for the increased tolerance was not discovered. In the present investigation evidence indicates that the tolerance of dogs to this drug is nonspecific since injections of large doses of Neo-Synephrine protected dogs against more than a surely lethal dose of epinephrine. Dogs tolerant to large doses respond to small doses of epinephrine with the same increase in blood pressure as was true before tolerance was developed. The portal venous pressure was markedly elevated by large doses of epinephrine. Casts made of the hepatic veins showed them to have gone into an extremely contracted state. A similar response was observed in the secondary branches of the pulmonary arteries of rabbits and cats following large doses of epinephrine. By maintaining the arterial blood pressure near the control level one dog in a series of 5 survived a rapid intravenous injection of 1.5 mg. of epinephrine per kg. In another series with the blood pressure controlled, 5 dogs out of 13 survived a rapid intravenous injection of 1.0 mg. of epinephrine per kg. In a series of 5 dogs with the arterial blood pressure uncontrolled none survived the latter dose.

Action of acetylcholine on cerebral cortex of cats and monkeys. CARL F. ESSIG (introduced by WADE H. MARSHALL). *Natl. Inst. of Mental Health, Natl. Insts. of Health, Bethesda, Md.*

Acetylcholine chloride in strengths ranging from 2-20% produces a localized depression of normal electrocorticographic activity when applied to the cerebral cortex. The depression shows little or no effect on areas remote to the site of application and can be obtained from any region on the dorsal convexity of the cerebral hemisphere. The local diminution in brain wave activity does not show an associated significant change in the cortical d.c. potential. The local nature of the brain wave changes and the lack of a significant direct voltage variation serves to differentiate the acetylcholine effect from the spreading depression of Leão. The depressant action may be followed by local high frequency large amplitude spikes often resembling grand mal seizure patterns. The appearance of such local signs of stimulation is not constant. Topically applied 1% eserine sulfate appears to accentuate the depressant action of acetylcholine. Atropine abolishes the depressant action of acetylcholine more consistently than it counteracts the spiking activity. In addition, atropine sulfate abolishes the depression in systemic blood pressure which follows the topical application of acetylcholine to the cerebral cortex. The blood pressure diminution increases with the strength of the acetylcholine and the volume applied. The decrease in blood pressure occurs without regard to the cerebral region to which the acetylcholine is applied and is accentuated by previous eserinization.

Suppression of ventricular ectopic rhythms by parasympathomimetic drugs. ANTONIO ESTANDIA,* A. SIDNEY HARRIS AND TEDROE J. FORD, JR.* *Dept. of Physiology, Baylor Univ. College of Medicine, Houston, Tex.*

Delayed ventricular tachycardia was produced in dogs by occlusion of the anterior descending artery as described elsewhere. Acetyl-β-methylcholine chloride (Mecholyl) in doses of 0.5 or 0.25 mg. per kg. subcutaneously markedly reduced the ectopic rate in all 6 dogs in which it has been tried. In some dogs completely normal rhythm has been restored for periods of a few minutes each and significant reduction of ectopic frequency has been maintained by a single dose for as long as 2 hours. Mecholyl has eliminated ventricular paroxysmal tachycardia while only insignificant changes in the sinus rate occurred. The effectiveness of Mecholyl has varied considerably in different animals. Carbamyl choline chloride (Doryl), 0.01 mg. per kg., was administered subcutaneously to one dog with a low frequency ventricular ectopic rhythm. The abnormal complexes disappeared for 1½ hours. Acetylcholine chloride, 0.4 mg. per kg., injected quickly by vein into a dog with low frequency ventricular ectopic activity, eliminated the abnormal complexes for about 2 minutes. These observations are regarded as evidence that ventricular premature systoles and tachycardia result from ectopic impulses and not from reentry. Since these vagomimetic substances shorten the refractory period and slow conduction, if they change it at all, they should make reentry phenomena more persistent. Since they eliminate abnormal complexes in many instances, this must be a result of diminished automaticity of discharging foci. This could be accomplished by

diminishing excitability or increasing accommodation to a continuing stimulus or both.

Strength and other physical properties of long bones of the inferior extremity. F. GAYNOR EVANS AND MILTON LEBOW (introduced by M. MASON GUEST). *Depts. of Anatomy and Engineering Mechanics, Wayne Univ., Detroit, Mich.*

Over 900 samples of compact bone from the human femur, tibia and fibula have been tested for tensile and shearing strength, modulus of elasticity, hardness and percentage elongation under tension. The samples were of standardized size and were tested in both the wet and dry condition. Air drying of the femoral samples increased their average tensile strength 31%, their modulus of elasticity 17% and their hardness 54%, but decreased their percentage elongation 45% and the shearing strength 18%. The percentage elongation under tension is directly proportional to the energy absorbing capacity (in lbs/in.^{3}) of the bone. Wet samples absorbed approximately 55% more energy before failing than did the dry ones. The tibia is the strongest of the 3 bones of the inferior extremity. The middle third of all 3 extremity bones is the strongest and the same is true of the lateral quadrant of the femur and tibia. The fibula was not tested according to quadrants. Variations of less than 1% in the density of the samples can be determined by the use of a radio-active beta-ray emitter (Strontium 90) and a Geiger counter. The results of these studies will also be reported as well as the fatigue strength of bone. Aided (in part) by a research grant from the National Institutes of Health, U. S. Public Health Service.

Hydrolysis of ethyl acetoacetate by normal rabbit bone marrow. JOHN D. EVANS (introduced by MORTON J. OPPENHEIMER). *Dept. of Physiology, Cornell Univ. Med. College, New York City and Dept. of Physiology, Temple Univ. School of Medicine, Philadelphia, Pa.*

An esterase of normal rabbit bone marrow hydrolyzes ethyl acetoacetate *in vitro* to ethyl alcohol and acetoacetic acid. When 0.8 mg. of ethyl acetoacetate is added to bone marrow cells suspended in Ringer's solution at 37°C. and at an initial pH of 7.3, approximately 90% is broken down in an hour. This is shown by *a*) liberation of carbon dioxide from a bicarbonate buffer, and *b*) analysis for ethyl alcohol. Physostigmine does not inhibit the action of this enzyme *in vitro*. Esterases in bone marrow also hydrolyze methyl acetoacetate, *n*-butyl acetoacetate, triacetin, and phenyl acetate at a rapid rate. On the other hand, rabbit marrow breaks down ethyl acetate, *n*-butyl acetate, ethyl propionate, ethyl *n*-butyrate, and *n*-butyl *n*-butyrate much more slowly. The esterase activity of bone marrow for ethyl acetoacetate is as great as that of liver, kidney or lung, and much greater than that of heart, muscle, spleen, pancreas or brain. The enzyme of ethyl acetoacetate hydrolysis may be of interest because of the importance of acetoacetic acid in metabolic processes.

Estimated duration of spontaneous neurogenic stimulation of the hypophysis, causing LH release in female rats. JOHN W. EVERETT (introduced by F. D. MCCREA). *Dept. of Anatomy, Duke Univ., Durham, N. C.*

In 4-day cyclic rats of our colony, spontaneous neurogenic activation of the hypophysis, inducing release of ovulating hormone, usually occurs between 2 and 4 P.M. on the day of proestrus. Either pentobarbital or atropine consistently blocks the activating mechanism when administered at 2 P.M., but rarely so at 4 P.M. The present study explores the intervening period. Each of 5 groups of 9–10 rats was given atropine sulphate (700 mg/kg., S.C.) at one of the following times: 2:40, 2:50, 3:00, 3:15, or 3:30. Autopsies were performed the next morning. Results were checked by serial sections of both ovaries, with particular reference to evidence of complete blockade or of partial activation. In 3/10 rats full activation had occurred before the 2:40 injection, while 2 others showed partial activation. In the remaining 4 series there was a more gradual increase in proportionate numbers activated. Among all 40 rats 9 cases of partial activation were found, representing stimulation in progress at the time of injection. Since the chance of interrupting a process is a function of its length, an estimate of duration was calculated: 26 minutes, with fiducial limits 14 and 52 minutes, respectively (2.5% level). The activating stimulus in rats, therefore, is not momentary as in rabbits, but proceeds gradually for possibly one-half hour. The results also indicate that secretion of ovulating hormone is quantitatively related to duration of stimulus. Stimulus and secretion may even proceed concurrently.

Effects of adrenal medullary hormones on blood and urinary chloride levels during water diuresis. W. J. EVERSOLE. *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

Subcutaneous administration of adrenaline (norepinephrine and epinephrine) in the rat stimulates chloride excretion and urine flow during water diuresis (STEIN AND WERTHEIMER. *J. Endocrinol.* 3: 356, 1944. GAUNT *et al. Endocrinology* 37: 136, 1945). A previous report from this laboratory indicated that the diuretic action of adrenal medullary hormone was not due to epinephrine but to the presence of nor-epinephrine (HORRES *et al. Proc. Soc. Exper. Biol. & Med.* 75: 58, 1950). The studies reported here on urinary electrolytes, collected over a period of one hour after injection of one dose of water and hormone, show that nor-

epinephrine caused a threefold increase in chloride concentration over the control level and hence greatly enhanced chloride excretion. Pure epinephrine had little or no effect on chloride excretion. Such findings indicate that the chloruretic action of adrenaline is due to its nor-epinephrine content. Injection of nor-epinephrine caused a slight increase in serum chloride whereas epinephrine failed to elicit such a response. Studies now underway are designed to explore the effects of adrenal medullary hormones on diuresis in terms of electrolyte, circulatory and renal mechanisms.

Post-natal development of water diuresis in rats. GERTRUDE FALK (introduced by E. F. ADOLPH). *Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.*

Newborn mammals are less able to compensate for water excesses and deficits than adults. This investigation was undertaken to trace the development of diuresis in rats after birth and to identify factors which influence the diuretic response. At birth there was no diuresis when 5% of the body weight of water was administered by stomach tube; but within a day there occurred a slow, delayed diuresis which, with subsequent ageing, began sooner and became greater. By 10 days of age a completely adult-like response was regularly obtained. At birth the urine tended to be hypotonic to the plasma and without appreciable change in concentration after water administration. With ageing and development of diuresis, urinary dilution increased. Absorption of water from the gastrointestinal tract was slower in infant rats than in adults, but exceeded the excretion rate at all ages. No differences were found in absorption rates in rats of 3–8 days of age, although important changes occurred in excretion rates. The number of nephra is said to double within 2 weeks after birth; but the number of nephra was not a limiting factor in the response, since no differences in results were found between unilaterally nephrectomized and control infant animals. Water diuresis was enhanced in rats of all ages by simultaneous administration of adrenal cortical extract, and inhibition with Pitressin was evident as early as 3 days of age. We infer that the factors involved in the development of this diuretic function are renal, hormonal and, possibly, hemodynamic and nervous.

Response in motility of non-innervated smooth muscle to *d*-tubocurarine chloride (curare). JOHN FERGUSON. *Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Nebr.*

A study was made of the effect of *d*-tubocurarine chloride (curare) on motility of non-innervated smooth muscle. The amnion of the developing chick was removed and suspended as a muscle strip in warmed aerated Sollmann-Rademaekers' solution. The motility was recorded on a smoked drum. This structure contains muscle tissue but is thought to be devoid of nervous elements of any description. Some preparations manifested spontaneous rhythmicity and changes in tonus. Only a rhythmic amnion was used for each application of curare. The response of different amnions was somewhat variable. Concentrations of pure powdered *d*-tubocurarine chloride in the bath ranging from 1:10,000 to 1:500 were used. A concentration of 1:1000 caused a decrease in tonus; but a concentration of about 1:600 was necessary to abolish both rhythmicity and tonus. In some instances it was still possible to obtain a response to stretch after a high concentration of curare. Modification of the tonicity of the solution in the bath by the addition of sodium chloride or glucose did not significantly affect motility in this muscle. A concentration of 1:3300 of curare in the bath appeared to antagonize the effect of acetylcholine bromide, an excitant of motility in this muscle, but the variability of response of the different amnions precluded making quantitative determinations.

Failure of 2,4-dinitrophenol to augment anaerobic glycolysis in rat cerebral cortex slices. JOHN FIELD, C. N. PEISS* AND VICTOR E. HALL. *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.*

Previous work from this laboratory indicates that both the heat and cold defense mechanisms are inactivated by 2,4-dinitrophenol (DNP). The phenomenon of general depression of both thermoregulatory mechanisms is a point in favor of the concept that the nitrated phenols have an uniformly depressing action, the augmentation of metabolic processes being a secondary feature. As part of a study of the action of various substances affecting body temperature (*Federation Proc.* 7: 33, 1948), we have determined the effect of DNP on anaerobic glycolysis in rat cerebral cortex slices. Anaerobic glycolysis was measured manometrically in a bicarbonate buffer at 37.5°C. Concentrations of DNP ranging from 0 to 2×10^{-3} M have essentially no effect on the glycolytic rate. Higher concentrations are inhibitory. The mean rate of anaerobic glycolysis in 72 control determinations was 7.5 μl./mg. dry wt. of tissue/hr. In view of the fact that other workers have demonstrated striking increases in anaerobic glycolysis due to DNP in other tissues, such as frog muscle, sperm and yeast, manometric determinations were checked by chemical analysis for lactic acid. The results obtained manometrically were confirmed by the lactic acid procedure. Furthermore, we were unable to demonstrate the aug-

menting action of DNP on anaerobic glycolysis in frog muscle. We are unable at this time to account for these discrepancies.

Deterioration of skeletal muscle due to contracture following local tetanus toxin injection. ERNST FISCHER AND HELEN V. SKOWLUND.* *Dept. of Physiology, Med. College of Virginia, Richmond.*

Rabbit gastrocnemii in contracture produced by local tetanus toxin injections were analyzed for fat, water and electrolyte content, for content in specific muscle proteins and for some enzymatic activities. The early onset of the considerable atrophy of these muscles is often masked by an initial edema during the so-called 'neurogenic' phase of the contracture. In the later phase of contracture, which cannot be reduced by nerve section or deep anesthesia, the intercellular potassium decreases considerably and is partly replaced by sodium. The loss in specific proteins such as myosin, acto-myosin and myogen, starts in the first phase, and at the same time myoalbumin content increses. Local tetanus contracture apparently has little effect upon the concentration of 'contractin,' the new muscle protein described recently for contracted muscles by Dubuisson. The various enzymatic activities of the muscles in contracture decrease more quickly than those protein fractions identified with these activities in the electrophoretic pattern of muscle extracts produced by various extraction methods.

Variations in the hyperdynamic cardiovascular response to stress among hypertensive subjects. JOHN T. FLYNN* AND STEWART WOLF. *Dept. of Medicine, New York Hospital-Cornell Med. Center, New York City.*

Variations in blood pressure, ventricular rate, peripheral resistance, and cardiac stroke volume were studied in 21 experiments on 17 hypertensive subjects at rest, after exercise and during discussion of significant personal conflicts, with the aid of the low-frequency critically damped ballistocardiograph. While this instrument is probably not suitable for comparative determinations of cardiac output from individual to individual or even from day to day, it can reliably reflect relative differences in cardiac output when it is used more or less continuously over a single experiment. Immediately after exercise all of the subjects displayed a characteristic hyperdynamic pattern including moderate elevation of arterial pressure attributable to an increase in stroke volume and cardiac output with a sharplylowered peripheral resistance. This same pattern was evoked by some subjects during discussion of personal problems, but others displayed a contrasting pattern characterized by a rise in arterial pressure attributable predominantly to elevated peripheral resistance and accompanied by no change or a fall in stroke volume and cardiac output. There were no consistent changes in ventricular rate during these observations. It is not possible from the determination of systolic and diastolic pressure or from the pulse pressure to ascertain which of these two patterns underlies an elevation of blood pressure The distinction, however, may be of considerable importance because available data indicate that blood pressure elevation by increasing cardiac output is well tolerated by the individual but that similar increases by the mechanism of peripheral vasoconstriction are more costly to the circulatory system in terms of energy required. Differential use of these patterns may clarify to some extent the puzzling observation that some subjects live for many years with a consistently elevated arterial pressure but with little evidence of circulatory damage, while in others rapidly progressive circulatory damage ensues following relatively short periods of arterial hypertension.

An insulin-free pancreatic extract capable of reducing the concentration of ketone bodies in the blood. PIERO P. FOA AND HARRIET R. WEINSTEIN.* *Dept. of Physiology and Pharmacology, Chicago Med. School, Chicago, Ill.*

Previous experiments indicated the possibility that certain insulin-free pancreatic extracts may contain a substance capable of lowering the concentration of Ketone bodies in the blood of fasting and diabetic dogs (*Am. J. Physiol.* 163: 711, 1950). These extracts, however, contained the hyperglycemic principle and the possibility that the lowering of the Ketone body might have been the result of the hyperglycemia had not been completely ruled out. It is known (and it was confirmed) that hyperglycemia produced by glucose is followed by a reduction of the Ketonemia, but this type of hyperglycemia is different from that produced by the hyperglycemic principle. A purified hyperglycemic factor (E. Lilly and Co., 1 mg/kg. i.v.; 4 depancreatized dogs) produced a marked (up to 70%) and protracted (up to 3 hr.) hyperglycemia, but the Ketonemia increased instead of decreasing. Epinephrine (0.02 mg/kg. i.v.; 5 depancreatized dogs) produced hyperglycemia without a decrease in Ketonemia. It is suggested that the extracts contain a substance capable of lowering the blood Ketones, and that this substance is different from insulin and from the hyperglycemic factor.

Cardiac outputs in normal dogs using cardiac puncture with fluoroscopic guidance. PAUL FORMEL,* THOMAS SEYMOUR,* SEYMOUR HORWITZ,* ANTONIO VERSACI* AND HAROLD C. WIG-

GERS. *Dept. of Physiology, Albany Med. College, Albany, N. Y.*

Repeated catheterization of the right heart in a given animal sooner or later renders the animal's veins unusable for other necessary venous injection and sampling procedures. Hence, in the chronic study of a given dog, we have taken recourse to the right ventricular puncture method to obtain mixed venous blood samples for cardiac output measurements. The 'blind' cardiac puncture, which others have described and which is generally considered hazardous and to be reserved for acute or critical situations, was found to be difficult, unreliable and too frequently injurious to the heart. The procedure, as now executed under fluoroscopic visualization, affords immediate entrance to the right ventricle for the spinal needle, with no significant cardiodynamic, respiratory or emotional disturbance to the quiet, trained dog. The latter is based upon observation as well as upon simultaneous determinations of heart rate, pulmonary ventilation and electrocardiograms. Mixed venous samples are withdrawn simultaneously with arterial samples from a previously inserted indwelling needle. Oxygen consumption is measured by connecting the face mask to a Benedict-Roth machine. Surgical explorations, electrocardiograms and arterial blood pressure readings have revealed no significant permanent damage as a result of repeated right ventricular punctures at weekly or greater intervals. Slight hemopericardium was seen in one instance. Cardiac output values for any given dog have been surprisingly consistent and compare favorably with those reported in the literature for trained dogs. The procedure and results will be discussed in detail.

Relationship between hypothalamic temperature and thermoregulatory effectors in unanesthetized cat. R. E. FORSTER, II AND T. B. FERGUSON (introduced by E. M. LANDIS). *Dept. of Physiology, Harvard Med. School, Boston, Mass.*

In order to estimate the importance and limits of the control of thermoregulation by the hypothalamic temperature, a small thermistor on the end of a fine glass needle was permanently affixed in the hypothalamus of 8 cats. The animals had recovered several days following operation and were maintained 6–67 days. Brain damage was minimal as judged both by physiological tests of temperature regulation and by microscopic sections on 3 animals. Hypothalamic temperature, as indicated by the thermistor, was from 0.52° above to 1.2°C. below rectal temperature and showed many irregular nonrhythmic variations as great as 0.5° C. without obvious cause. Deep peritoneal sodium pentobarbital anesthesia eliminated the small variations in hypothalamic temperature and rendered the animals relatively poikilothermic, whereas light anesthesia had no consistent effect. In general, rest tended to lower hypothalamic temperature; exercise or excitement to increase it. In 5 cats, panting, whether produced by radiant heaters or by hot air, occurred whenever the hypothalamic temperature rose above a 'threshold' range. When the hypothalamic temperature dropped below this range following the intragastric introduction of cold water through a nasal catheter in one of these animals, panting ceased in spite of a continued external heat load. Two animals showed no relationship between hypothalamic temperature and panting and were considered 'reflex' panters. In 5 cats tested, thermoregulatory vasodilation and vasoconstriction usually occurred without any apparent causal relationship to hypothalamic temperature. These data are felt to be consistent with the hypothesis that in most unanesthetized cats, panting is primarily controlled by hypothalamic temperature, while thermoregulatory vasomotor reactions, within the limits tested, are not.

Reduction in glomerular activity induced by 'cigarette smoke apnea' in rabbits. ROY P. FORSTER. *Dept. of Zoology, Dartmouth College, Hanover, N. H.*

Respiratory movements were found to be inhibited when such olfactory irritants as tobacco smoke were presented to rabbits. When apnea was induced in this manner for 10 seconds out of each minute over a period of 10 minutes it resulted in lowering glomerular filtration rates 50% and in reducing the number of active glomeruli (glucose Tm) by 40% during this period of partial respiratory arrest. Subsequently the number of functioning glomeruli rose to control values immediately upon resumption of normal breathing. However, somewhat reduced glomerular filtration rates persisted for 30 minutes or more after the end of the period of partial apnea. Urine flows were drastically lowered from control values of 2.0 ml/minute to 0.14 ml/minute. This antidiuretic effect was observed even after maximal urine flows had been obtained by superimposing osmotic and water diuresis. Slowing of the heart rate and other cardiac irregularities accompanied these alterations in kidney function. This intermittency of glomerular activity is viewed as a vestigial phenomenon present in only some of the terrestrial mammals, and is perhaps elicited in these forms solely during certain states of stress.

Effect of adrenalectomy on blood protein-bound iodine. W. C. FOSTER, B. CALESNICK AND N. H. SMITH (introduced by J. F. MCCLENDON). *Depts. of Physiology and Pharmacology, Hahnemann Med. College, Philadelphia, Pa.*

Very few animal experiments have been reported

on the relationship between the thyroid and adrenal glands. Since the blood protein-bound iodine has proven to be a measure of thyroid activity, a study was undertaken to determine the relationship of these two glands by this method. The blood protein-bound iodine was determined in control and adrenalectomized dogs, with and without pellets of desoxycorticosterone acetate, by the method of McClendon and Foster. Two stage adrenalectomies were performed, and pellets implanted. These pellets were removed at varying intervals. In one dog DCA was not implanted as a pellet, but administered daily. The postoperative course in the dogs was followed closely by a study of blood levels of potassium, sodium, chlorides, glucose, vitamin C, and cholesterol. Adrenalectomy was found to reduce the blood protein-bound iodine, despite the implantation of the pellets. Removal of the pellets produced a further lowering of the blood protein-bound iodine. Daily administration of desoxycorticosterone elevated the level of blood protein-bound iodine.

Effects of d.c. polarization on spinal reflexes. KARL FRANK (introduced by F. J. MULLIN). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Following the suggestion of Gerard (*Anesthesiology*, Jan. 1951), that causalgia is related to altered potential fields and hypersynchronization within the spinal cord, hyperirritable cats have been prepared by injection of alumina cream under the cord dura (KENNARD. *J. Neurophysiol.* May 1950). The electrical state of such cords will be studied, especially under polarization. Preliminary control observations on spinal reflexes of normal cats confirm Renshaw's finding (*J. Neurophysiol.* 3, 1940) on the effects of d.c. polarizing currents; a 20 ma. current between ventral root and cord can augment (root positive) or depress (root negative) the monosynaptic reflex response recorded from that ventral root. The changes are shown to be due, however, solely to local alteration of spike amplitude by electrotonus under the pickup electrodes. Varying the separation between the polarizing and pickup electrodes on the ventral root gives a space constant for the effect of polarization on spike height of about 5 mm. (A small polarizing current between pickup electrodes is an effective means for making a normally diphasic root potential purely monophasic.) Greater polarizing currents (ball, needle or plate electrodes in many arrangements) within the cord do alter reflex responses. With a mouth-to-anus current of 4 ma., monosynaptic responses are selectively abolished (mouth positive) or augmented (mouth negative). The induced potential gradient is about 20 mv/cm. in an axial direction and uniform across the cord. A 10 ma. current (50 mv/cm.) alters multisynaptic responses as well. Similar effects with needle electrodes in the cord require damagingly large currents (0.4 ma.). Selective depression of the monosynaptic response is apparently due to failure of afferent impulses to reach the motoneurons.

Isolation of C^{14}-labelled purines and pyrimidines from biological materials. JACQUES R. FRESCO* AND ALFRED MARSHAK. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

Biological synthesis may serve as a means of preparing compounds of high specific activity, which are useful in tracer studies. Adequate proof is required of the chemical purity of the compounds isolated. The following procedure attempts to meet such needs. *Thiobacillus thioparus* is grown in a medium with $C^{14}O_2$ (5% C^{14}) as the sole carbon source. The acid-soluble and lipid portions are extracted from the recovered bacteria. The residue is homogenized in water by freezing and thawing, and subsequently extracted three times with 5% trichloroacetic acid at 90° C. for 30 minutes to separate proteins and nucleic acids. Boiling for one hour decomposes the trichloroacetic acid in the supernatant. The pH is then brought to zero with hydrochloric acid, and the solution boiled for one hour to liberate the purines. The hydrolysate is subjected to the countercurrent procedure of Tinker and Brown. These gross separations minimize possible contamination in the final steps of purification. The resulting adenine and guanine fractions are separately precipitated as silver salts, which are then dissolved in hydrochloric acid and individually subjected to ion-exchange chromatography. The pyrimidine nucleotide fraction is hydrolyzed with 12 N perchloric acid and similarly chromatographed. The specific activity of each base is then determined, after which aliquots are subjected to paper partition chromatography with at least three solvent systems. The specific activity should remain constant. Analysis of aliquots by the isotope derivative method gives further confirmation of purity. Data on recovery will be presented.

Gastric reabsorption of hydrogen ion as mechanism reducing the acidity of parietal secretion. M. H. F. FRIEDMAN. *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

Experiments were performed on two dogs with a Thomas-Pavlov pouch and one dog with a Heidenain pouch. The pouches comprised about 15% of the acid-secreting area of the stomach. The pouch stomas were made so as to retain liquids without loss over a period of 3 hours. Each animal was also equipped with cannulated fistulas of the main stomach and duodenum. Studies were made only when the gastric glands were in a state

of secretory rest, as determined by the reaction of the main stomach. Acids and salts, singly and in combination, were introduced into the pouch as solutions of known sulfate, chloride, sodium, potassium, and hydrogen concentrations. The volumes used were either 15 or 25 ml. The pouch was drained at intervals and an alloquote was obtained for analyses. The remainder was reintroduced into the pouch after restoration to the original volume by addition of solutions of appropriate composition. The data to be presented offer an explanation of the observed differences in the acidity of the gastric juice obtained at different rates of secretion. The results are consistent with the view that the primary acidity of the juice following its secretion by the parietal cell is altered by exchange of hydrogen ions, through reabsorption, for sodium ions, through diffusion from the serum.

Rate of disappearance of digitoxin from the blood of man after its parenteral administration. MEYER FRIEDMAN, SANFORD O. BYERS AND RENÉ BINE, JR.* *Harold Brunn Inst. for Cardiovascular Research, Mount Zion Hosp., San Francisco, Calif.*

By means of the embryonic duck heart method, as little as 0.1 μg. of digitoxin could be detected in a cc. of blood serum. This sensitivity has allowed a quantitative study of the amount of digitoxin in human serum and its rate of disappearance following the intravenous administration of digitoxin. Four subjects were given 1.2 mg. of digitoxin by vein. Immediately following injection, the average content of digitoxin was 0.15 μg/cc. of serum (range: 0.1–0.2 μg/cc.). Sixty minutes later the serum content of digitoxin remained relatively unchanged (0.1 μg/cc. of serum). At the end of 120 minutes, the serum of 2 subjects still contained 0.1 μg. of digitoxin/cc. of serum, but the serum of the remaining 2 subjects contained no detectable digitoxin. At the end of 180 minutes none of the patients' sera contained detectable digitoxin. These results suggest that digitoxin disappears very much slower from blood than the previously tested digitalis glycoside, Lanatoside C.

Some temperature relations of fish. F. E. J. FRY (introduced by EDGAR C. BLACK). *Dept. of Zoology, Univ. of Toronto, Toronto, Ontario, Canada.*

Under this title is presented an account of a number of unpublished observations on lethal temperatures, temperature preferenda and optimum temperatures for continuous activity in fish which have been accumulated in the Ontario Fisheries Research Laboratory in recent years. It is pointed out that there is no necessary connection between the lethal temperature of a species and its optimum temperature. There is, however, usually a close correspondence between the optimum for activity and the final temperature preferendum.

Effect of ultrasound on central nervous system of frogs. W. J. FRY* AND V. J. WULFF. *Univ. of Illinois, Chicago.*

Ultrasound incident on the dorsal surface of frogs above the lumbar enlargement of the spinal cord produces paralysis of the hind limbs. The time of exposure to ultrasound of frequency of 1 mc. required to paralyze frogs varies with sound amplitude, hydrostatic pressure and initial temperature of the preparation. The reciprocal of the exposure time required for paralysis (all frogs paralyzed), when plotted as a function of sound amplitude gives rise to a linear relation. This linear relation indicates that a threshold amplitude exists below which no paralysis will occur regardless of the duration of exposure. Similar data obtained from frogs exposed to ultrasound while subjected to a hydrostatic pressure of 13 atm. (sufficient to prevent cavitation) yield a linear relation whose slope is smaller and threshold amplitude is slightly greater than the relation obtained under atmospheric pressure. Temperature as a factor in producing paralysis must be considered in three categories: *1*) high average temperature level caused by absorption of acoustic energy and measured with a thermocouple imbedded in the spinal cord; *2*) rapid time rate of change of temperature also caused by acoustic absorption; and *3*) minute regions of high temperature caused by the absorption of ultrasound at interfaces. Experiments with cooled frogs demonstrate that paralysis can occur with maximal temperatures of 15°C. Analyses based on the linear relations previously discussed indicate no correlation between paralysis and time rate of change of temperature. Theoretical considerations indicate that conversion of acoustic energy into heat at cellular interfaces result in temperature differences between interface and bulk of material of less than 0.1°C.

Renal hypertrophy in potassium-deficient rats. FREDERICK A. FUHRMAN AND ADELAIDE BROKAW.* *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.*

In the course of an investigation of carbohydrate metabolism in potassium-deficient rats it was observed that the kidneys of the deficient animals appeared to be abnormally large. Consequently further experiments were carried out to determine the nature and extent of this hypertrophy. Male albino rats (Holtzman) 4 weeks old were fed a diet containing 0.3 mM/100 gm. potassium.

Litter mate controls were pair-fed the same basal diet with K added to a concentration of 11 mM/100 gm. Two K-deficient animals died after 9 days so the experiment was terminated at that time. The K-deficient animals gained an average of 1 gm. in weight. The kidneys of the control group (10 animals) averaged 0.96 gm. fresh weight and 0.21 gm. dry. Those of the K-deficient group averaged 1.73 gm. fresh and 0.33 gm. dry. The increased fresh weight of the kidneys of the K-deficient animals is thus partially accounted for by the increased water content (420 gm/100 gm. dry weight compared to 350 gm/100 gm. dry weight in the controls), but even on a dry weight basis the kidneys of the K-deficient animals were 57% heavier. The adrenal glands of the K-deficient animals weighed more than those of the pair-fed controls in 7 out of 10 pairs. We have observed increase in the weight of adrenal glands in other series of K-deficient animals which were not pair-fed, and it has been reported by others (cf. GARDNER *et al.*, *J. Lab. & Clin. Med.* 35:592, 1950). (This work was performed under a contract between the Office of Naval Research and Stanford University.)

Frequency localization in cochlear nucleus. R. GALAMBOS, JERZY E. ROSE AND J. R. HUGHES.* *Psycho-Acoustic Lab., Harvard Univ., Cambridge, Mass., and Dept. of Psychiatry, Johns Hopkins Univ., Baltimore, Md.*

Anatomical studies describe neurons projecting from basilar membrane to cochlear nucleus in a complex but orderly way. Our objectives were to discover whether physiological responses to pure tones occur in some pattern at the cochlear nucleus, and, if so, whether this pattern corresponds to that predictable on anatomical grounds. Twelve cochlear nuclei in 11 cats under Dial-urethane were examined. Glass micropipettes (3–5 micra) were introduced in steps of 50 or 100 μ. The response area and characteristic frequency were determined at each step. The characteristic frequency is that narrow band of sound frequencies which produces a response at the weakest intensity that excites (GALAMBOS AND DAVIS. *J. Neurophysiol.* 6:39, 1943.) Characteristic frequencies for single units and for groups of units are equally useful in studying localization. Two aspects of the results will be discussed. *1*) The characteristic frequency drops from high to low as the electrode penetrates from above in approximately the frontal plane. In one experiment an orderly and uninterrupted drop from 25–11.5 kc. was observed for 23 steps over a distance of 1.10 mm. *2*) Single units with a wide range of characteristic frequencies were isolated, of which one at 140 cps was studied in detail. The behavior of this low frequency unit was similar in every regard to that of single auditory units previously described at this level.

'Alveolar air' and arterial blood gas tension studies in normal and chronic lung disease patients. MORTON GALDSTON, BRY BENJAMIN* AND MARYLIN HUREWITZ.* *New York Univ. Research and Med. Services, Goldwater Memorial Hosp., and Dept. of Medicine, New York Univ. Med. School, New York City.*

Collection of arterial blood samples for calculation of mean alveolar air pO_2 might be obviated in pulmonary function studies if it can be established that end-expiratory air represents mean alveolar air. Comparisons between pCO_2 arterial blood and end-expiratory air (drawn from just beyond the mouth) and pO_2 of calculated mean effective alveolar air and end-expiratory air were, therefore, made in normal subjects and in some suffering from chronic lung disease. In 47 observations on 8 normal subjects, mean pCO_2 in end-expiratory air was 39 mm. Hg $\pm$ 1.2 and mean pO_2 102 mm. Hg $\pm$ 2.4. In 9 additional observations arterial blood was drawn simultaneously. The mean pCO_2 in end-expiratory air was 39 mm. Hg $\pm$ 1.7 and 39 mm. Hg $\pm$ 0.9 in arterial blood; while mean pO_2 end-expiratory air was 102 mm. Hg $\pm$ 1.9, mean effective alveolar air 100 mm. Hg $\pm$ 2.3 and in arterial blood 95 mm. Hg $\pm$ 4.8. In contrast, in abnormals, similar comparisons showed that end-expiratory air does not represent mean alveolar air. In 30 observations on 21 abnormals mean pCO_2 in end-expiratory air was 33 mm. Hg $\pm$ 6.6, arterial blood 42 mm. Hg $\pm$ 9.3; mean pO_2 in end-expiratory air was 109 mm. Hg $\pm$ 8.9, mean effective alveolar air 98 mm. Hg $\pm$ 3.8, arterial blood 76 mm. Hg $\pm$ 5.5. Breathing 14, 18, or 25% oxygen did not alter the differences. These tension differences substantiated by other measurements (total dead space/tidal air $\times$ 100, alveolar air nitrogen after 7 minutes oxygen inhalation, functional residual air/total capacity $\times$ 100) indicate primary disturbances in gas distribution which may occasionally be accentuated by insufficient washing out of the anatomical dead space.

Development of cardiac reflex to time intervals. W. HORSLEY GANTT, W. A. GAKENHEIMER* AND ALBERT STUNKARD.* *Pavlovian Lab., Phipps Clinic, Johns Hopkins Univ., Baltimore, Md.*

Using two tone signals (T256+ with shock and T512− without shock) for shock to paw in dogs, excitatory and inhibitory cardiac conditional reflexes (crs) can be formed even more easily than motor crs (withdrawal of paw). With regular alternation of T256+ and T512− (5-sec. duration) at 2-minute intervals, differentiation of cardiac crs (heart rate) develops after 50 or more repeti-

tions of the tones. A time cr appears in the cardiac rate *first* (after 150–300 repetitions), *later* in respiration and leg movements but the cardiac cr is the most reliable and delicate measure of the time reflex. The heart rate (HR) is greatest during T256+ and shock (100–200), drops within 15″ (60–100), is lowest within 10″ after inhibitory tone (512−), and rises gradually during next 50″ before excitatory tone (256+). Only after 500 repetitions is there anticipatory anxiety evident by whining. Brain lesions (hemidecortication plus limbic lobe extirpation on opposite side) impair or destroy time reflex but not differentiation measured by cardiac crs to the two tones. Omission of the inhibitory signal (T512−) halfway between the two excitatory signals does not destroy the cardiac cr to time. Thus the cardiac crs are not only more delicate measures of adaptation to a situation than are the somatic movements, but they give a clear-cut picture of adaptation to time intervals not obtainable through the other usual measurements.

Deflection of electrogram of heart muscle. Solomon Garb (introduced by McKeen Cattell). *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

The electrograms of isolated papillary muscles of the cat's right ventricle were recorded on a Dumont, Model 279, cathode ray oscilloscope. The normal pattern consisted of an upright R deflection followed by an upright T deflection. The T was inverted by anoxia, rapid rate of stimulation, and injury. The same situation existed when two leads were recorded simultaneously from different areas of the same muscle, and when unipolar electrograms were recorded on a direct-writing EEG. This suggests that the inverted T deflections recorded on muscle strips by other investigators resulted from the anoxia or injury to which the preparation was subjected. Since the T deflection is normally in the same direction as the R deflection in a simple muscle strip under relatively physiological conditions, it is unlikely that it can be ascribed to an opposite electrical process. It seems probable that the T deflection is not produced by membrane repolarization.

Effects of beta rays on mammalian nerve action potentials. Edgar L. Gasteiger (introduced by Berry Campbell). *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Radiation effects can be well quantitated in studies on nerve action potentials, and in such studies new and interesting information about axons can be obtained. Action potentials of excised saphenous, sural, and vagus nerves of cats were recorded during beta irradiation. A small calibrated bulb of radon used as a source of beta rays was applied at a point between stimulating and recording electrodes, and recordings were made until conduction of the action potentials had ceased. Upon determining the per cent change of nerve activity as a function of the product of exposure time and millicuries of radon, several observations were made. The latency of the conducted impluse increased as the irradiation increased. All fiber groups decreased in activity in accordance with a sigmoid survival curve, but the rate of decrease was different for various groups. Specifically, in the A fiber sub-groups the order of increasing sensitivity was alpha, gamma, and delta. At higher temperatures the changes were greater and the sensitivity differential was reduced.

Blood pressure changes following explosive decompression of monkeys to extreme altitudes. Samuel Gelfan and Attie Yvonne Werner.* *Aeromedical Research Unit, Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

Cardiovascular responses following sudden decompression to extreme altitudes and recompression at free fall rate are the result of mechanical effects of ambient pressure changes and the rapidly induced anoxia. The following arterial pressure changes, recorded by strain gauge continuously throughout the experiment, occur. *1*) Momentary rise due to initial Valsalva effect at the instant of decompression. *2*) Immediate lowering due to carotid sinus and other reflex reactions to sudden rise in pressure. Complete recovery within 10 seconds or less if *factors 3* and *4* do not supervene. *3*) Additional lowering due to maintained impairment of venous return from lower half of body caused by positive intra-abdominal pressure, inevitably developing at altitudes above about 44,000 feet. Extent of this effect is related to altitude and recovery is prompt upon recompression. *4*) Following, superimposed upon and exacerbated by *3* is the precipitous drop due to the rapidly induced total anoxia at altitudes above 52,000 feet, even in 100% oxygen. The mean anoxic drop in arterial pressure is to about 45% of control level. *5*) Time of recovery is related to altitude and barometric pressure during free falling descent at which adequate reoxygenation of blood occurs. Contrary to previous reports, the pulse pressure, throughout the period beginning immediately upon decompression, never disappears, even at final ambient pressures of 20–26 mm. Hg (80,000–75,000 feet).

Physiology of arousal reaction. E. Gellhorn and M. Bernhaut.* *Lab. of Neurophysiology, Univ. of Minnesota, Minneapolis.*

Experiments were performed on numerous cats and monkeys at different stages of barbiturate anesthesia on the effect of various forms of afferent stimulation on cortical and hypothalamic

potentials. These effects were correlated with signs of arousal such as spontaneous movements, pupillary dilatation and ocular movements. The effectiveness to rouse the animal increased from optic to nociceptive stimulation with acoustic and proprioceptive stimuli in an intermediary position. Optic stimuli did not produce either signs of arousal or excitation of the cortex outside the optic projection area. Acoustic and proprioceptive stimuli caused different degrees of cortical excitation varying between activation of specific projection areas and generalized cortical excitation depending on the depth of anesthesia. Other conditions being equal cortical excitation was more widespread and signs of arousal more pronounced with proprioceptive than with acoustic stimuli. Nociceptive stimuli (electric stimulation of the sciatic) produced generalized cortical and arousal reactions in surgical anesthesia at which proprioceptive stimulation caused cortical effects in the sensori-motor area only. Moreover, it was found that only arousal reactions and generalized cortical excitation were associated with hypothalamic excitation (cf. GELLHORN AND BALLIN. *Am. J. Physiol.* 146: 630). Large diencephalic lesions destroying the hypothalamus abolished generalized cortical reactions without interfering with excitation of specific projection areas. The arousal reaction is thought to involve the hypothalamic-cortical system. (Aided by a grant from the office of Naval Research.)

Activation of human profibrinolysin. EARL B. GERHEIM, ANDRE M. WEITZENHOFFER* AND GEORGE R. LOGWOOD.* *Div. of Research, Univ. of Detroit School of Dentistry, Detroit, Mich.*

To the list of activators for human profibrinolysin, T. A. Loomis (*J. Lab. & Clin. Med.* 36: 82, 1950), has added filter paper. This phenomenon was demonstrated with Human Plasma Fraction I. We have been unable to confirm these results with any of several brands of filter paper. In addition, filter paper in our experiments has not shown any activator properties with a potent purified human profibrinolysin. Yet this profibrinolysin assayed in excess of 30 U/mg. of ether-acetone dried protein on activation with streptokinase-streptodornase of Lederle, using the fibrinolytic technic of E. C. Loomis and colleagues (*Arch. Biochem* 20: 444, 1949). There was absolutely no evidence of fibrinolysis over a period of 48 hours in the presence of more than 30 U of profibrinolysin with filter paper in the quantities of the fibrinolytic assay method. Filter paper was not an activator for the profibrinolysins of dog, cow or horse prepared in this laboratory.

Inhibition of spinal motoneurons during increase of pH and inflation of carotid sinus. ROBERT GESELL, A. S. DONTAS* AND D. C. PETERS.* Dept. of Physiology, Univ. of Michigan, Ann Arbor.

The nature of inhibition was studied by observation of electrical and functional activity of phrenic motoneurons. A d.c. amplifier connected with emerging phrenic rootlets was used to register slowly oscillating neurocellular currents which spread along neuraxons by electrotonus and the normally transmitted spike or action potentials of inspiratory activity. Negativity of nerve cell body with respect to neuraxon showed typical rise and fall with each inspiratory and expiratory phase, increase and decrease of negativity being associated with an increase and absence of spike potential respectively. Inhibition was attended by abolition or reduction of slow oscillations of electrotonic current, abolition or reduction of spike potential and fall of basic level of negativity of nerve cell bodies. Reduced electrotonic potentials at supernormal pH are thought to result from chemical withdrawal of synaptic potentials due to supernormal activity of cholinesterase. Such withdrawal should equalize polar potentials, reduce flow of neurocellular currents and inhibit nerve cell activity. Reduced electrotonic potentials during inflation of carotid sinus is also theoretically attributable to equalization of polar potentials resulting in this case from augmented impingement of nerve impulses on the inhibitory pole of the neuron. The consequent reduction of neurocellular currents would, as above, explain inhibition of nerve cell activity.

Photometric assay of plasma heparin. R. B. GIBSON, T. L. CARR,* SAUL GREEN* AND W. M. FOWLER* (with the assistance of IMOGENE JENSON). *Depts. of Biochemistry and Internal Medicine, State Univ. of Iowa Hosp., Iowa City.*

Plasma is diluted with an equal volume of M/15 phosphate buffer pH 6.2 and precipitated by repeated addition of octylamine hydrochloride. The precipitate, obtained by centrifuging, is washed in saline, dissolved in warm alkaline saline and reprecipitated with zinc sulfate solution. This precipitate is extracted at 65°–70°C. with phosphate buffer (plus saline) at a pH of 7.4. The supernatant is transferred to another centrifuge tube and toluidine blue O (or azure A) solution added. After several hours, or overnight, a red precipitate forms. This is centrifuged, washed with water and dissolved in alkaline alcohol. A heparin standard and a dye blank reference tube are prepared. The heparin content is read, spectrophotometrically at 530 mμ. Transmittancy plotted semi-logarithmically against concentrations is a straight line graph. Values for normal plasma heparin are usually between 0.08 and 0.12 mg. %. Plasma levels following i.v. administration of

sodium heparinate and of 'depo-heparin' are in accord with the corresponding clotting times. Observations on heparin plasma levels in certain blood dyscrasia, in inhibition of heparin effect with toluidine blue and protamine sulfate, and in histamine and proteose shock are being studied.

Effect of acetylcholine and of vagal inhibition on the demarcation potential of the turtle heart. A. S. GILSON, JR. *Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

Gaskell (*J. Physiol.* 8: 404, 1887) reported positive variation of the demarcation potential of heart muscle during vagal stimulation. We have investigated this phenomenon using hearts of large slider turtles (P. elegans) excised with attached vagal trunks permitting stimulation remote from the heart. Calomel electrodes with wool wicks served as leads to a circuit including potentiometer and a sensitive galvanometer for indicating zero balance. Leads were taken from a variety of points on atrial epicardial and endocardial surfaces. Vagal stimulation and solutions of acetylcholine bromide (ACh) were used. Results were irregular and unsatisfactory from a quantitative standpoint. Qualitatively in no case was the Gaskell interpretation confirmed. If any potential change occurred in response to ACh or vagal action there was increased local negativity. The base of the atria which has been used previously as a region for study showed slight if any inhibition to ACh or vagus action. This may have led to misinterpretation of results in earlier experiments on turtles. It is known that the characteristic inhibitory actions of vagus and of ACh alike are absent in the turtle ventricle and in the embryo before innervation of the heart. Such facts and the results reported above would be consistent with the hypothesis that the cardio-inhibitory action of vagus fibers and of ACh both occur by intermediation of ganglion cells and, further, that the postganglionic fibers may produce their inhibitory effect by an action akin to cathodal rather than to anodal polarization.

Sympathetic stimulation and critical closing pressure. FRANK GIRLING (introduced by ALAN C. BURTON). *Dept. of Biophysics, Univ. of Western Ontario, London, Ontario, Canada.*

Since Claude Bernard, at about the middle of the last century, demonstrated that stimulation of the cervical sympathetic could produce constriction of the blood vessels of the rabbit ear, surprisingly little quantitative work has been done regarding the effect, on the blood vessels, of electrical stimulation of the vasomotor nerves. The only paper encountered, dealing at all quantitatively with this subject, has been one by Grutzner and Heidenhain in 1877. It has been shown in previous papers presented to this society, by Drs. Burton and Nichol, that a critical closing pressure does exist, and that vasoconstrictor drugs cause not only constriction but an elevation of this critical closing pressure. It seemed desirable, in view of this, to investigate the effects of electrical stimulation of vasomotor nerves. This was attempted first in the hind limb of the rabbit, but, due to inherent difficulties, a modified Gaddum-Kwiatkowski ear preparation was finally used. With this preparation, and double cannulation of the common carotid artery, it was possible to control the blood flow through the ear by means of varying the frequency of stimulation. Like vasoconstrictor drugs, electrical stimulation causes not only constriction of the vessels, but also an increased critical closing pressure. It could well be that, in that type of hypertension associated with increased sympathetic activity, the increase in pressure is required, because of an increased critical closing pressure. The margin of available pressure, i.e. the pressure above that which is required to produce any flow at all, may be no greater than in the normal.

Measurement of skin penetration by use of subcutaneous probe-counter in conjunction with radioisotope labelled material. HYMAN R. GITTES,* E. ROSS HART* AND AMEDEO S. MARRAZZI. *Toxicology Section, Med. Div., Army Chemical Center, Md.*

Continuous measurement of skin penetration by radioactive DFP has been described by Hart, Fleisher and Marrazzi (*Federation Proc.* 8: 300, 1949). Since the method calls for a superficial collecting vein draining a given region, it is not readily applicable to all animals or to humans. We have, therefore, experimented with the Ballantine Laboratories probe-counters inserted subcutaneously within a radius of several centimeters of the site of application on the skin of DFP synthesized with P^{32}. Rise of activity in the subcutaneous counters indicates the proximity of the material resulting from penetration through the skin. This is continuously recorded by rate meters activating ink writers. The systemic concentration of material is measured simultaneously by another probe inserted in the symmetrical and contralateral region where no material has been placed on the overlying nearby skin. Comparison of the two methods (subcutaneous and cutaneous counters) and analysis of the factors are presented.

Evidence for presumptive relationship of Castle's anti-anemic intrinsic factor to glandular mucoprotein of human gastric juice. GEORGE B. JERZY GLASS, LINN J. BOYD, MICHAEL A. RUBINSTEIN, CHESTER S. SVIGALS AND JACQUELINE E. CHEVALLEY (introduced by STEWART WOLF).

Dept. of Medicine, New York Med. College, Flower and Fifth Ave. Hosps., and Metropolitan Hosp. Research Unit, New York City.

In earlier studies on the mucous substances of human gastric juice Glass and Boyd reported properties of one of them, gastric mucoprotein, which resembles intrinsic factor. Both substances contain protein moieties, are non-dialyzable, filtrable, non-precipitable with trichloracetic acid, precipitable with ammonium sulfate, and can be stored in solution at low pH and 5°C. Like intrinsic factor mucoprotein is present in normal gastric juice and occurs in increased concentration in gastric juice of patients with duodenal ulcer. Both derive in man from the glands of the gastric fundus in which atrophic changes occur in pernicious anemia. It has been found that gastric mucoprotein is absent or present only in traces in patients with pernicious anemia. In the present study 5 patients with proven pernicious anemia were given daily oral doses of 20–30 μg. of vitamin B_{12} to which was added, after a control period, 50–100 mg. of gastric mucoprotein from human sources. In one case in partial relapse no hematopoietic response was obtained. In this case only a weak response occurred following subsequent parenteral administration of vitamin B_{12}. In 4 cases in complete relapse, however, a typical hematopoietic response was obtained after mucoprotein was added to small oral doses of vitamin B_{12} for 7–16 days. This consisted in a sharp rise in reticulocytes (15–31%), followed by a significant increase in RBC and Hb., disappearance of megaloblasts from bone marrow, and complete clinical remission when treatment was sufficiently prolonged. In no case was a comparable response observed following oral administration of the small doses of vitamin B_{12} alone. The data suggest that glandular mucoprotein from the human stomach contains Castle's intrinsic factor, or is identical with the latter. Further studies on this problem are in progress.

Changes in blood pressure in response to presumably and to potentially painful stimuli. F. R. Goetzl and C. Bien.* *Inst. of Med. Research, Permanente Fndn., Oakland, Calif.*

An analysis of literature has led to the suggestion that vasomotor responses to presumably painful stimulation may be dependent in part upon the perception of pain. The present experiments were conducted in order to test the correctness of that suggestion. Electrical stimuli of variable voltage were applied to the tooth pulp of curarized rabbits and cats, conscious and anesthetized. The animals had been prepared so that changes in blood pressure could be recorded as well as changes in volume of leg, spleen and kidney. The results obtained indicate that the stimulus used, if of sufficient intensity to produce any vasomotor response, produced a rise in blood pressure accompanied by corresponding changes in volume of organs in the conscious, and a fall in blood pressure accompanied by corresponding changes in volume of organs in the anesthetized animal. Since tooth pulp is known to contain no receptors other than pain receptors the observations are interpreted to indicate that the rise in blood pressure following stimulation of tooth pulp was dependent upon the perception as pain of the stimulus employed. Pharmacological analysis (epinephrine, cocaine, ergotamine, Dibenamine) revealed that the rise in blood pressure mentioned resulted from sympathetic discharge. It is suggested that this sympathetic discharge is dependent upon the perception of pain and that it may be useful for objective demonstration of perception as pain of a potentially painful stimulus.

Studies on metabolism of analogous mammary tumors. A. Goldfeder, H. Albaum and L. Eisler.* *Biology Dept., New York Univ. and Dept. of Hospitals, New York City.*

Previous investigations by one of the authors (A. G.) have shown that two mouse mammary adenocarcinomas, histologically very similar, exhibited a significant difference in their respiratory and overall metabolic activity *in vitro*, as determined by the Barcroft-Warburg manometric technic. The O_2 uptake and aerobic glycolysis of the relatively faster growing tumor was about 3 times as high as those of the slower growing one. The metabolic rate of these tumors corresponded roughly to the growth rate. Further investigations on phosphorylated intermediates were carried out on actively growing tumors by the use of the freezing technic and enzymatic methods. The components determined included total acid soluble phosphorus, inorganic orthophosphate, lactic acid, glycogen, glucose-1-phosphate, fructose-6-phosphate, fructose-1-6-diphosphate, phosphoglyceric acid, coenzyme I, adenylic acid, adenosine diphosphate, adenosine triphosphate, and phosphocreatine. The more rapidly growing mammary tumor showed significantly higher levels of glucose-1-phosphate, adenosine triphosphate, and a lower level of inorganic phosphorus. These findings suggest that a higher level of energy is available for vital functions and are in agreement with other observations made on this tumor. Investigations are in progress to determine the entire glycolytic cycle in the dynamic state of the faster and slower growing tumors applying tracer methods, using radioactive phosphorus. These results will be presented. Observations along these lines, made on other mammary adenocarcinomas, are now accumulating and will also be reported.

Failure of vitamin B_{12} to reverse aminopterin inhibition in the fruit-fly Drosophila melanogaster. E. D. GOLDSMITH AND MORRIS H. HARNLY.* *Dept. of Histology, College of Dentistry, and Washington Square College of Arts and Sciences, New York University, New York City.*

Incorporation of aminopterin (Lederle), 4-amino-pteroylglutamic acid, in the fruit-fly basal food ration, a yeasted Pearl's synthetic medium, resulted in a retarded rate of development and in many cases failure of the Drosophila to complete the life cycle. Survival values were inversely proportional to the concentration of aminopterin employed. Supplementation of the Pearl's medium containing 5.0 mg.% of aminopterin with 5, 50, 100, 400 and 800 μg.% of crystalline B_{12} (Merck) produced no improvement over the emergence values obtained when the antagonist was used alone. No evidence of toxicity was observed in the controls to which vitamin B_{12} was added in concentrations up to 800 μg.% It has been reported previously that pteroylglutamic acid and desoxyribonucleic acid (Schwarz) have proved very effective in annulling the antagonist action of 4-amino PGA in Drosophila; more recently, the Citrovorum Factor (Lederle) has also been found to revese the aminopterin inhibition (Goldsmith and Harnly). Other factors and combinations are being studied.

Comparison of the inhibitory effect of sodium azide on the in vitro respiration of resting and stimulated tissues of normal and hyperthyroid rats. M. S. GOLDSTEIN (introduced by E. B. ASTWOOD). *Ziskind Research Labs. of the New England Center Hosp. and the Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Male albino rats were rendered hyperthyroid by feeding a diet containing 2% desiccated thyroid. After 10–14 days BMR determinations indicated rises of 20–30% in oxygen consumption. *In vitro* oxygen consumption was measured in the Warburg apparatus and determinations were made on diaphragm, anterior abdominal wall muscles, and on salivary gland slices. Tissues of normal rats were compared with tissues of hyperthyroid rats both in the resting state and while being stimulated. The muscle was stimulated by applying intermittent electric stimuli across the fluid in the vessel by means of electrodes sealed into the glass walls, thereby inducing contraction of the muscle, and the salivary gland slices were stimulated by the addition of acetylcholine. In each case sodium azide was tipped onto the tissue after a control period. In all tissues of the hyperthyroid series the *in vitro* increase in oxygen consumption from thyroid feeding was of the same order of magnitude as seen in the living animal. The concentration of sodium azide employed (0.002 M) was without effect on the oxygen consumption of normal resting tissues during the first hour but promptly reduced the hyperthyroid values to those of the normal controls. The increase in oxygen consumption accompanying stimulation of the various tissues was similarly reduced to that of the resting controls by addition of the inhibitor. It would seem that the increases of oxygen consumption above the resting normal value induced either by thyroid hormone excess or by increased activity of the tissue are both sensitive to inhibition by sodium azide, possibly pointing to involvement of a common metabolic site.

Resumption of development in chick embryos after solidification in liquid nitrogen. FEDERICO GONZALES* AND BASILE LUYET. *Dept. of Biology, St. Louis Univ., St. Louis, Mo.*

Primitive streak blastoderms were removed from the eggs and transferred to chick Ringer's. They were then sectioned transversally about 0.5 mm. behind the Hensen's node and the anterior portion was *1*) partially dehydrated by immersion in a hypertonic solution (30%) of ethylene glycol; *2*) frozen in liquid nitrogen; *3*) thawed by immersion in chick Ringer's at about 40°; *4*) placed on the surface of an albumin-extract-saline-agar medium where further development could be followed (method of Spratt). Blastoderms so treated grew 'tails' from the cut end of the primitive streak. Several showed development of head fold and brain vesicles. Heart beats were observed; one heart continued beating for more than 48 hours. Thus embryonic cells lose neither their ability to grow nor their differentiation and organization power on being frozen.

Effects of adrenalectomy and hypophysectomy upon red cell fragility. ALBERT S. GORDON AND HERBERT MEGEL.* *Dept. of Biology, Washington Square College, New York Univ., New York City.*

It has already been reported (PILIERO, LANDAU AND GORDON. *Science*, '50. MEGEL AND GORDON. *Endocrinology*. In press) that the resistance of whole blood of the rat to hypotonic lysis is increased after adrenalectomy. The factors responsible for this change in fragility are present in plasma and operate, most likely, by becoming adsorbed upon the red cell. The resistance of whole blood to hypotonic saline is also augmented following hypophysectomy and to a greater extent than that seen in the adrenalectomized animal. Washing with isotonic saline tends to remove the inhibitory factor from the red cells. By this procedure more of the factor can be separated from the cells of the adrenalectomized than from those of hypophysectomized rats. Although adrenalectomized rat plasma is more effective than normal

rat plasma in affording extra resistance to washed cells from normal rats, plasma obtained from hypophysectomized animals lacks this property. Addition of charcoal to the plasma of adrenalectomized rats results in removal of the inhibitory factor from the plasma. The quantity removed is proportional to the amount of charcoal added. Some of the material adsorbed on the charcoal can be eluted with acetone. Evaporation of the acetone yields a water-soluble precipitate which is capable of increasing the resistance of washed cells of normal rats towards hypotonic saline. Experiments are in progress to determine the nature and mechanism of action of the plasma inhibitory complex.

Hydraulics of mitral insufficiency. RICHARD GORLIN AND LEWIS DEXTER (introduced by GEORGE W. THORN). *Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Five studies of mitral insufficiency and stenosis were performed in man by the technique of cardiac catheterization. Cardiac outputs (CO) were measured by the direct Fick principle. Mean pulmonary 'capillary' pressures were measured as an index of left atrial pressure. Brachial artery mean systolic pressures were measured as an index of left ventricular mean systolic ejection pressure. Diastolic filling and systolic ejection periods per beat were measured on the brachial arterial tracing. An indirect approach to the quantitative aspects of mitral insufficiency was made via hydraulic principles. The two basic principles employed were: 1) Area = Rate of valvular flow/ Constant $\times \sqrt{\text{pressure gradient}}$; 2) Fm = Fr + CO; where Fm = total left ventricular output, liters/minute; Fr = mitral regurgitant flow, liters/minute. The following formulae were derived for estimating the cross-sectional area of the regurgitant mitral orifice (MRA) and the amount of regurgitant blood flow (Fr):

$$\text{Fr} = \text{DFP} \times \text{MVA} \times 31 \sqrt{\text{"PC"} - 5} - \text{CO};$$

$$\text{MRA} = \text{Fr/SEP} \times 44.5 \sqrt{\text{BA}_{sm} - \text{"PC"}};$$

where DFP = diastolic filling period per minute, sec./min.; MVA = 'forward' mitral valve area, cm.^{2}; 31 = empirical constant; 'PC' = pulmonary 'capillary' pressure, mm. Hg; 5 = assumed left ventricular diastolic pressure, mm. Hg; SEP = systolic ejection period per minute, sec./min.; 44.5 = empirical constant; BA_{sm} = brachial artery mean systolic pressure, mm. Hg. Studies were made at rest, during exercise, and on recovery. The area of the mitral valve was obtained at the time of mitral valvuloplasty in three patients and was assumed for purposes of demonstration to equal 2 cm.^{2} in the other two. Calculations checked in all instances. These formulae possess many practical weaknesses as regards the accuracy of calculations in man, although the dynamics of the disease may be demonstrated. The major errors lie in 1) inability to measure directly left ventricular pressures and flow periods; 2) inability to check at autopsy calculated and measured regurgitant orifice areas; and 3) the necessity thus far of using surgical rather than autopsy measurements of mitral valve areas.

Convulsions induced in psychotic patients by oxygen inhalation at increased ambient pressures (motion picture). R. A. GOULD, C. J. LAMBERTSEN, R. H. KOUGH, M. W. STROUD, J. H. EWING AND F. A. FREYHAN (introduced by J. E. ECKENHOFF). *Depts. of Pharmacology, Medicine and Psychiatry, Univ. of Pennsylvania, Philadelphia, Pa.*

A 16-mm. colored motion picture, approximately 15 minutes running time shows in detail the procedure and progressive manifestations occurring in subjects during the inhalation of oxygen at high partial pressure (OHP). It covers the interval from before the onset of symptoms to the conclusion of the post convulsive recovery phase. The exposures are carried out in a pressure chamber accommodating the subject and 4 investigators. The film is part of a broad study of the effects of OHP. As part of this investigation, the possibilities of oxygen convulsions as a new form of convulsive psychiatric therapy are being studied. The subjects of this film are young, chronic schizophrenic men unresponsive to other forms of convulsive therapy.

Influence of ACTH on sodium and potassium content of human mixed saliva. B. GRAD (introduced by O. F. DENSTEDT). *Allan Memorial Inst., McGill Univ., Montreal, Quebec, Canada.*

The influence of ACTH on the sodium and potassium content of saliva was investigated in each of 4 patients by collecting samples of unstimulated mixed saliva before each meal and at bedtime for several days before, during and after ACTH therapy. The samples of saliva were centrifuged and aliquots of the supernatant were diluted; determinations were made by means of a flame photometer, and sodium concentrations/potassium concentration ratios were calculated. In addition, once-daily measurements of the urinary 17-ketosteroid excretion and circulating eosinophil counts were made in one of the patients. In each of the patients studied, ACTH reliably reduced the sodium/potassium values from the pretreatment control level. In 3 of the patients the reduction in the sodium/potassium ratio during ACTH therapy was due both to a reduction in the sodium concentration and an increase in the potassium concentration of the saliva, while in the

fourth patient the effect was seen mainly in the reduction of the sodium concentration. Each patient showed specific times of the day when he was more sensitive to this effect of ACTH than at other times. In some patients this was before breakfast, and in others before dinner or before retiring.

Effect of x-radiation on the growth of *Torulopsis utilis*. BARBARA M. GRANT* AND F. G. SHERMAN. *Biology Dept., Brown Univ., Providence, R. I.*

Standard sized innocula of a 24-hour culture of *Torulopsis utilis* (ATCL # 8206) were introduced into 10 ml. portions of a synthetic medium (devised by Atkins and coworkers) containing 0.1 gm. $(NH_4)_2SO_4$ per liter (ATKINS *et al. Biochim. et Biopys. Acta.* 3: 692, 1949). Medium and cells were irradiated together in soft glass tubes with doses of 5, 10, 15 and 20 thousand r at a rate of 1260 r per minute. The source of radiation was a therapy tube operated at 100 kv. and 10 ma. without an additional filter. The cultures were then aerated by shaking in special culture flasks immersed in a 30° water bath. Growth was followed for 24-36 hours by determining optical density at intervals. Irradiation results in a reduction of total growth approximately proportional to the dose. It was demonstrated by reducing the innoculum size of non-irradiated cultures to the number of colony-forming cells remaining after irradiation that the reduction of total growth was not due to a decrease of the number of viable cells present after irradiation. Irradiated cultures reached a maximum growth rate later than non-irradiated cultures and the maximum rate attained was less than that of the controls.

Thermoregulatory thresholds in thyroidectomized rabbits. RONALD GRANT. *Dept. of Physiology, Stanford Univ., Stanford, Calif.*

Berde (*Hungarica Acta Physiol.* 1: 52, 62, 1947) claims that the body temperature of thyroidectomized rodents "mounts more rapidly and higher in hot surroundings than does that of normal animals", due to loss of the 'thermothyrins' thought to depress metabolism in hot environments. The effects of high environmental temperatures (ET) on rectal temperature (RT), respiratory rate (RR), ear hyperemia and oxygen consumption (OC) were studied in male rabbits before and after thyroidectomy. Heat tolerance is decidedly improved after thyroidectomy. RT rises less and more slowly and stabilization of RT can be achieved at higher ET. In moderate heat thyroidectomized rabbits stabilize RT with less hyperthermia and less polypnea. RT thresholds for onset of ear hyperemia and polypnea are unaffected by thyroidectomy but long standing thyroprivia seems to impair ability to achieve extreme polypnea. High ET has no direct 'reflex' effect on either ear hyperemia or RR. Despite a rise in RT both normal and thyroidectomized rabbits show decreased OC in hot environments. This is due to decreased random activity and presumably accounts for the ability of both normal and thyroidectomized animals to stabilize RT in hot environments despite saturation of heat loss mechanisms. These results do not necessarily contradict the existence of thermothyrins but do contradict the improbable postulate that thyroidectomized animals are less resistant to high ET. A general dulling of thermostatic responses is suggested by the low levels of polypnea reached in hot environments and the characteristic moderate hypothermia of thyroidectomized rabbits at moderate ET despite ability to shiver strongly on appropriate stimulation.

Erythropoietic activity of bone marrow demonstrated in vivo using colchicine. WILSON C. GRANT. *Dept. of Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

An attempt has been made to measure empirically the functional state of erythroid bone marrow in the adult rat. Since the first evidence of increased erythropoietic activity is probably mitotic division, use was made of the alkaloid colchicine which arrests mitosis and thus permits an enumeration of easily recognized mitotic figures which accumulate in the marrow during a given time. Colchicine (S. B. Penick & Co.) in aqueous solution, 0.01–0.20 mg/100 gm. body weight, was injected subcutaneously and rats were sacrificed 1–8 hours later. Femoral marrow suspended in homologous serum was smeared and stained with Hematoxylin-Eosin-Azur. Many mitotic figures were evident in almost all types of marrow elements at doses above 0.01 mg. and duration greater than 1 hour. As a preliminary attempt to quantitate the response, the number of erythroblasts in pro-metaphase was expressed as a percentage of the total nucleated cells. In the marrow of uninjected rats, mitotic figures were rare making counting impractical. The percentage of mitotic figures rose with the dosage of colchicine up to 0.1 mg. and with time to 8 hours. Using 0.05 mg. of colchicine and 5 hours duration as a standard procedure, the erythroid mitotic percentages of 12 normal rats ranged from 2.5–4.8, average 3.6. Fifteen rats subjected to moderate hemorrhage (2 cc/100 gm.) were given colchicine 1 or 2 days later and revealed a range of 3.8–12.0%, average 6.9%. Six rats discontinuously exposed over 3 days (18 hours total) to 400 mm. Hg atmospheric pressure were given colchicine and presented a range of 5.6–8.5%, average 7.0%. Increased erythroid mitotic activity following two well known

erythropoietic stimuli, anoxic anoxia and hemorrhage, occurs before a well established erythrocyte or reticulocyte response can be detected in the peripheral blood.

Action of enzymes on potassium loss from red cells. JAMES W. GREEN (introduced by W. W. SWINGLE). *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.*

Washed, rabbit red cells were incubated at 35° C. for periods up to 36 hours with proteolytic and lipolytic enzymes and the potassium losses from the cells studied at intervals during this time. Phosphate buffers were used and the studies made at several pH values. The results indicate that commercial lipase has a marked effect in lowering the potassium concentration of the cells while trypsin has a negligible effect. Mylase P, a mixture of proteolytic and dephosphorylating enzymes appears to produce a retention of potassium within the cells.

17-Ketosteroid excretion during therapy of diabetic acidosis. LAWRENCE GREENMAN,* T. S. DANOWSKI, M. ROSENBLOOM* AND JOHN HOLT.* *Univ. of Pittsburgh School of Medicine; Children's and Presbyterian Hosps., Pittsburgh, Pa.*

Urinary 17-ketosteroids were measured by Holtorff and Koch's method in 12 patients, 5–17 years old, at 2- to 7-hour intervals during therapy of diabetic acidosis, and then during 24-hour intervals in convalescence. Keto acids were destroyed in the preliminary hydrolysis of the urine. Blood eosinophils were counted in 5 patients. One patient died 2 hours after admission; 5 were males and 4 newly discovered diabetics. All were overbreathing on admission with serum HCO_3 ranging from 2.7–9.8 mEq/liter (av. 5.8). 17-Ketosteroid excretion ranged from 0.43–2.45 mg/hour (av. 0.87) during acidosis in those who recovered, and eosinophil counts from 0–31/cu. mm. (av. 7). The patient who died excreted only 0.28 mg/hour; a value significantly lower than any in the survivors during a similar period. Urinary 17-ketosteroids gradually decreased to amounts normal for the maturity of each subject, 0.09–0.56 mg/hour (av. 0.31) when recovery was established. These latter figures represent 24-hour values of 2.3–14.3 mg. (av. 7.4); while the early values ranged from 2–5 times higher. The elevated 17-ketosteroid excretion and eosinopenia with subsequent return to normal suggest increased activity of the adrenal cortex during recovery from diabetic acidosis, and contrasts with the smaller and perhaps inadequate response of the patient who died.

Use of ether in prevention of cyclopropane-epinephrine-induced arrhythmias. E. M. GREISHEIMER, DOROTHY ELLIS, M. J. OPPENHEIMER AND H. W. ROBINSON. *Dept. of Physiology, Temple Univ. School of Medicine, Philadelphia, Pa.*

A small amount of ether added to a cyclopropane-oxygen mixture decreases the incidence of arrhythmias due to epinephrine. Using a modification of Kety's method (cyclopropane analyzed at about 1°C. and ether at about 41° C.) it was determined that ether exposures up to 500 mg. minutes will not prevent the appearance or reduce the duration of the arrhythmias of epinephrine-cyclopropane. Exposures above 500 cause reduction or absence of arrhythmias. This is true even when the cyclopropane levels are held constant and not reduced as is clinically the case when ether is turned in. The absolute levels of cyclopropane appear to be unrelated. Oxygen and carbon dioxide levels of whole blood are unchanged. Serum and whole blood potassium are not significantly different under cyclopropane-ether. Inorganic phosphorous is regularly increased by cyclopropane alone. This rise persists when ether is added. This elevation of phosphorous can be shown not to be due to epinephrine or pentothal in these experiments. Cyclopropane alone or with added epinephrine often is accompanied by ventricular beats which will develop integrated pressures sufficient to open the pulmonary valve but not the aortic. An analysis by use of three simultaneous unipolar leads places the origin of most of the cyclopropane-epinephrine ectopic beats in the upper anterior right ventricle. All these cardiovascular phenomena are prevented or have their latency increased and duration reduced by ether. Fibrillation is imminent when epinephrine is used without ether.

Influence of portal circulation on restoration of liver after partial hepatectomy. JOHN H. GRINDLAY AND JESSE L. BOLLMAN. *Div. of Exptl. Medicine, Mayo Fndn., Rochester, Minn.*

Following removal of standard lobes of the dog's liver, equivalent to 70% of the total liver, the liver is rapidly restored and the original weight is approximated in 30 days. Partial hepatectomy performed after diversion of the caval blood to the portal vein, i.e. portal-caval anastomosis and ligation of the vena cava above the anastomosis, permits complete restoration of the liver. Similar hepatectomy following or simultaneous with diversion of the portal blood from the liver as in Eck's fistula is followed by only slight restoration of the liver. Ligation of the vena cava in dogs with portal-caval anastomosis and ligation of the portal vein permits almost normal restoration of the liver after partial hepatectomy. After placement of slightly constricting cellophane bands on the portal vein or vena cava, fibrosis develops around the band which usually occludes the vein within a few weeks. Partial hepatectomy after band oc-

clusion of both portal vein and vena cava at its entrance into the liver is followed by almost normal restoration of the liver. When the portal vein alone is occluded only slight regeneration of the liver occurs after partial hepatectomy. From these observations it would appear that occlusion of the vena cava just below the liver benefits the nutritive condition of the liver deprived of portal blood.

Right and left part-electrocardiogram of the fetal heart. FRANZ M. GROEDEL AND MAX MILLER.* *Madison Ave. Hospital, New York, N. Y.*

Electrocardiograms obtained with semi-unipolar technique from the surface of the right and left ventricle of the human heart, respectively, show two different patterns, identical to the right and left part-electrocardiogram from the outer chest wall, as described in 1933 by Groedel. Feti, delivered by caesarian section and kept alive in warm saline solution, soon after the operation were electrocardiographically studied—limb leads and chest exploration. Thereafter the thorax was opened, the exploring electrodes placed upon the right and left ventricle, respectively, and both electrograms recorded simultaneously with the indifferent electrode at the right arm. We show only the findings in the youngest specimen, examined in this way, a four-month-old fetus. The right and left electrocardiograms from the chest wall were of low voltage; the P waves well recognizable; conduction time 0.09 second. The right initial complex consisted of one positive wave of very low voltage; finer differentiation was impossible. The left initial complex showed a higher R and small S wave. Both T waves were positive. Duration of the electrical systole was 0.36 second. The right and left electrogram from the surface of the right and left ventricle, respectively, were about twice the voltage of the chest leads. In the right eg, P waves were not recognizable; the initial complex consisted of a small R and a still smaller S wave; T was positive. The left eg showed a bifid P wave; conduction time was 0.16 second. The initial complex consisted of a high R and small S wave; no Q was present. The left T was inverted; the duration of the electrical systole was 0.36 second. The voltage of the right R was about one third that of the left R. The peaks of the left R and S waves were formed about 0.15 second later than that of the right R and S respectively.

Effects of stimulation of lumbar sympathetic nerves on longtitudinal bone growth in dogs. GLENN GULLICKSON, JR.,* W. G. KUBICEK AND F. J. KOTTKE. *Div. of Physical Medicine, Univ. of Minnesota, Minneapolis.*

The response in bone growth of the hind limbs to unilateral stimulation of the lumbar sympathetic chain was studied in young puppies. The left lumbar sympathetic chain was stimulated chronically with 3 cps sinusoidal alternating current, using implanted silver electrodes, from 23–70 days. The criterion of response to stimulation was a decrease in the skin temperature of the left hind leg. When there was no response the dogs were killed, the bones of both hind limbs dissected out, and the longitudinal length of the femur, tibia, and metatarsals determined for the right and left sides, the right hind limb being used as a control. The individual lengths of the bones were summated and the difference between the summated lengths of the right and left hind limbs determined. Ten dogs, considered to have been adequately stimulated, all showed a decrease in length on the left as compared with the right, the difference varying from 0.5–3.5 mm., the average 2.8 mm. Most of the shortening took place in the metatarsal bones. Six puppies with left lumbar sympathectomy all had increases in the length of the left hind limb as compared with the right of 2.7–14.0 mm., the average 6.5 mm. The maximum difference in length of the two hind legs found in 7 controls was 1 mm., the average 0.2 mm. The findings indicate that imbalance of the sympathetic nervous system may be a definite factor in unequal bone growth of the legs. The decrease in leg length on the stimulated side was probably due to a decreased blood supply to the growing bone as a result of chronic vasoconstriction.

Subjective appraisal of comfortable brightness relationships. SYLVESTER K. GUTH AND A. A. EASTMAN (introduced by JOSEF BROŽEK). *Lamp Dept., General Electric Company, Nela Park, Cleveland, Ohio.*

An extensive series of investigations was conducted to determine the relationships among the various factors which govern whether sources of brightness are comfortable or uncomfortable. Ten representative observers made appraisals of the sources. The factors studied included the size of the source, its position in the visual field and the adaptation brightness. The criterion was the visual sensation at the borderline between comfort and discomfort. The observers adjusted the brightnesses of the sources until this sensation was produced. The sizes of sources were from 0.001–0.13 steradian and the adaptation brightness ranged from 1–100 footlamberts. Sources were located in various positions of the upper right quadrant of the binocular visual field. The relationship between source brightness and size was found to be a variable one, proportionately greater reductions in brightness being required as the size of the source was increased. The relationship for the range of adaptation brightnesses used was a linear logarithmic one. It was found that a source of constant size and brightness required a greater

angular displacement from the line of vision along the horizontal than along the vertical meridian. The results indicated a complex relationship among these factors which could be expressed by the empirical equation, $B = 108\ PF^{0.44}(Q^{-0.21} - 1.28)$ where B is the brightness at the borderline between comfort and discomfort produced by a source having a size Q and a position index P, viewed amid an adapting field brightness F.

Recording pulse pressures through skin in dogs. ARTHUR C. GUYTON. *Dept. of Physiology and Biophysics, Univ. of Mississippi School of Medicine, University.*

At a preliminary operation, the carotid artery of a dog is elevated from its bed and sutured in a fold of skin so that the apex of the fold, with the artery contained therein, protrudes approximately one-half inch. To record the pulse pressures a special screw clamp attachment is placed over the artery in such a manner that pulsations of the artery are limited by metal walls of the device in all aspects except two. First, the clamp does not impede the free communication of blood between the carotid artery within the clamp and the proximal end of the artery outside the clamp. Second, one wall of the clamp is a loose rubber membrane which is bounded on the opposite side by a solid-walled fluid chamber. When just sufficient fluid is placed in this chamber almost to collapse the artery, but not quite, the pressure pulses are transmitted to the fluid chamber. Recordings made through a lead tube from this chamber to an electromanometer cannot be distinguished from recordings made directly from a #18 needle. The correct amount of fluid in the chamber is determined by beginning with too little fluid and gradually increasing the amount. Special changes in the characteristics of the recordings, as have been determined from a series of experiments, enable the operator to adjust for the appropriate amount of fluid.

Peripheral circulatory factors as determinant of cardiac output. ARTHUR C. GUYTON AND JEFF W. HARRIS.* *Dept. of Physiology and Biophysics, Univ. of Mississippi School of Medicine, University.*

In 13 open-chest, heparinized dogs a flowmeter and a primer pump were inserted between the great veins and the right auricle. It was found that vigorous activity of the primer pump could not increase the right auricular pressure more than an average of 1.33 mm. Hg, the cardiac output more than an average of 44%, and the arterial blood pressure more than an average of 20%. The reason for this was that the peripheral veins collapsed before greater values than this could be reached. It is reasoned that the negative venous pressure of the thorax is more or less equivalent to a primer pump because of the external suction tending to dilate the heart and great veins. Furthermore, this suction is considerably greater than the 1.33 mm. Hg increase in auricular pressure which is necessary to enable the heart to pump all possible blood which may be returned to it. Therefore, it appears that the cardiac output in normal animals is almost entirely, if not entirely, determined by factors in the peripheral circulatory system which affect venous return rather than by cardiac factors.

A spectroscopic study of oxidation by tyrosinase of certain proteins, protein hydrolysates and synthetic derivatives of tyrosine. WARD J. HAAS,* IRWIN W. SIZER AND JOHN R. LOOFBOUROW.* *Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.*

Kinetic ultraviolet absorption spectroscopy at 298 and 77°K has been applied to the study of the action of tyrosinase on tyrosine and on proteins and synthetic compounds containing tyrosyl groups. Evidence is obtained that the oxidation of susceptible substrates proceeds through the 3–4 dihydroxyphenylalanine (dopa) group as an intermediate. Only in the case of tyrosine is there formed a typical hallochrome intermediate and, as the end product, the characteristic black insoluble melanin pigment. The oxidation by tyrosinase of susceptible proteins (insulin, pepsin) and protein partial hydrolysates is characterized by an overall increase in optical density; by a pronounced decrease in the ratio of maximum to minimum absorption; and by the development of a shoulder in the 325 mμ region. Differences in the oxidation of tyrosine and of proteins by tyrosinase are ascribed to the fact that some tyrosyl groups in proteins are refractory because they do not have free phenolic hydroxyl groups. Other differences are related to the fact that in proteins both the tyrosyl amino and carboxyl groups are usually combined in peptide bonds. Studies of the action of tyrosinase on certain synthetic derivatives of tyrosine (p-methyoxyphenylalanine, tyrosine ethyl ester, N-formyl tyrosine, and N-formyl tyrosine ethyl ester) are consistent with this interpretation. In the case of those derivatives in which carboxyl, amino, or both, groups were combined, the action of tyrosinase was parallel in most respects to its action on proteins and protein partial hydrolysates.

Complete reversibility of inhibition of hyaluronidase by serum. ZAREH HADIDIAN. *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

When partially purified bull testes hyaluronidase is incubated with normal serum *in vitro*, there is an initial inhibition followed by a partial recovery of hyaluronidase activity (*Biochem. J.* 42: 266, 1948). Hyaluronidase preparations varying a hundred-fold in activity show this phenomenon, indicating the presence of a factor which inactivates the non-specific inhibitor of hyaluronidase. A factor pres-

ent in hyaluronidase preparations of bacterial origin and snake venom was shown by Haas (*J. Biol. Chem.* 163: 89, 1946) to destroy the serum inhibitor when the latter was exposed to it *before* acting on hyaluronidase. Venom of Agkistrodon piscivorus will, when added to a serum-hyaluronidase mixture *after* the serum has exerted its maximum effect, restore the activity of hyaluronidase completely within a few hours. There remains no doubt that the reaction between hyaluronidase and the non-specific serum inhibitor is completely reversible; and this reversal may be produced by a factor closely associated with hyaluronidase. Characteristics of the inactivation of serum by venom to be described support the claim that it is an enzymatic reaction. In view of the confirmed importance of magnesium in the hyaluronidase-serum reaction (*J. Biol. Chem.* 172: 347, 1948; *Proc. Soc. Exper. Biol. & Med.* 70: 524, 1949; 74: 279, 1950), above procedures were carried out in the presence of excess magnesium. The activation of the inhibitor, however, is not a unique property of magnesium, but is demonstrated by at least one other ion, cobalt, which is effective maximally at a concentration of 0.25 mM/1.

Inhibition of glycogenesis in muscle by alloxan and restitution by insulin. DAVID E. HAFT* AND I. ARTHUR MIRSKY. *May Inst. for Med. Research, Cincinnati, Ohio.*

Although it has been demonstrated that alloxan can influence the activity of many isolated enzyme systems, its effect on such systems in the intact cell is unknown. For such reasons, the influence of alloxan on glycogenesis in the isolated rat diaphragm was studied. Except where otherwise noted, one or both hemidiaphragms were incubated for 5 minutes with alloxan or with saline adjusted to the same pH as the alloxan. After two washings with phosphate-saline buffer, the tissues were incubated for 90 minutes in the buffer containing glucose. The difference between the glycogen concentrations of the hemidiaphragms served to indicate the effect of pretreatment. In some experiments, one of the two hemidiaphragms was incubated with insulin.

PRETREATMENT		GLYCOGEN DIFFERENCES mg/gm. (mean ± S.E.)	
Slide A	*Slide B*	*Without Insulin*	*With Insulin 0.02 U/ml. in B*
None	none	+0.003±0.2	+1.37±0.21
None	saline pH 3.5	−0.36±0.11	
Saline pH 3.5	alloxan 0.05 M	−0.95±0.12	+0.25±0.12
Alloxan 0.05 M	alloxan 0.05 M		+0.82±0.2

Acid-saline pretreatment produced some diminution of glycogenesis ($0.01<P<0.02$), but a more marked further diminution was produced by alloxan ($0.001<P<0.01$). A restitution to levels obtained with acid-saline and untreated muscles followed incubation with insulin. No statistically significant difference between the untreated muscles and those treated with alloxan and then incubated with insulin was observed, ($P = 0.05$). Thus, alloxan inhibited glycogenesis and insulin caused a restitution to the non-alloxanized state.

Effect of purified growth hormone on growth of islets of Langerhans in hypophysectomized, intact, and glucose-injected rats. R. E. HAIST AND B. KINASH.* *Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada.*

The injection of a purified growth hormone preparation (Armour and Co.) into intact Wistar rats led to an increase in the weights of the islets of Langerhans as estimated by the method of Haist and Pugh. The injection of this material into hypophysectomized rats caused an increase in islet weights as compared to those of uninjected hypophysectomized animals. However, the injected hypophysectomized rats ate more than the uninjected animals and gained more weight. Since caloric intake greatly influences the islet growth, this may be an important factor in this experiment. Growth hormone, injected into rats receiving a continuous injection of glucose, did not appear to hinder the increase in islet weight which normally results from the glucose injection. These experiments support the view that pituitary growth hormone stimulates rather than depresses the islets of Langerhans.

Protection of mice from lethal effects of x-radiation with intravenous injection of methylene blue. B. VINCENT HALL (introduced by DOUGLAS E. SMITH).* *Biological and Med. Div., Argonne Natl. Lab., Chicago, Ill. and Dept. of Zoology, Univ. of Illinois, Urbana.*

In 12 independent experiments, over 600 male and female mice were given varied x-ray doses of 500–900 r (200 kv., hvl 0.98 mm. Cu, target distance 11 inches, dose rate 104 r/minute). Half of the mice were injected intravenously 2–8 minutes before irradiation with methylene blue solutions whose dye content varied from 2.5 to 10.0 mg/cc. Dosages of 25, 37.5, and 50 μg. of dye/gm. mouse were used. Varying the amount of dye injected had little effect on protection, yet it appears that the concentration of the dye solution is a factor influencing survival after irradiation. Excellent protection was found consistently in 8 experiments employing solutions of 0.25–0.5%, whereas only 1 of 4 experiments using 1% solutions yielded statistically significant protection. The maximum protection obtained was 50–60% differential between the percentage with methylene blue 30-day sur-

vival of dye protected and control mice. Mice injected with methylene blue after irradiation were not protected. The percentage of 303 dye-pretreated mice surviving 30 days after irradiation was nearly double that of 310 dye-free irradiated controls. The methylene blue protection may possibly result from the formation of methemoglobin which induces hypoxia of the tissues sufficient to ameliorate the lethal action of irradiation, or methylene blue may have a more direct action on tissues affording protection. Experiments are being continued which may help answer these questions.

Production of hypertension in the rat by parabiosis. C. E. HALL AND O. HALL.* *Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.*

Hypertension has been produced in parabiotic rats by inducing perinephritis in one of them; by total nephrectomy of 1, or extirpation of 3 of the 4 kidneys of the pair; or by treating one of the pair with desoxycorticosterone acetate. Certain evidence gathered by the last technique suggested that hypertension might be produced by parabiosis *per se*. Accordingly, the hypothesis was tested. Twenty-eight pairs of female rats placed in parabiosis and allowed to drink 1% sodium chloride were studied for periods up to 45 days. In 12 pairs hypertension as great as 222 mm. Hg developed in one of the partners, the other remaining normotensive or displaying occasional hypotension. Both partners in the remaining 16 pairs remained normotensive. Eight pairs, of which 4 were hypertensive, died before the 28th postoperative day. The remaining 20 pairs were sacrificed at periods varying from 29–45 days. Renal and cardiac hypertrophy, nephrosclerosis, periarteritis nodosa and myocarditis were prominent in the hypertensive animals and were never observed in normotensives. Twelve single control female rats remained normotensive. The experiment indicates that hypertension may be produced in parabiotic rats without further surgical intervention in about 40% of the pairs. The lesions observed are identical to those produced by desoxycorticosterone in the rat, and horse serum or sulfonamide allergy in the rabbit. Further studies are in progress to determine whether the observed syndrome is due to stress, allergy or a combination of both. (Supported by a grant from the American Heart Association.)

Hemoglobin oxygen affinities in the blood of various vertebrate animals. F. G. HALL. *Duke Univ. School of Medicine, Durham, N. C.*

Hemoglobin oxygen affinities from bloods of various fishes, amphibians, reptiles, birds and mammals have been determined. Correlations are made with the environments in which these forms were found. It is concluded that the oxygen hemoglobin dissociation curves of the bloods studied offer little evidence of phylogenetic relationships. It would appear that the specific hemoglobin oxygen affinity is hereditarily well fixed in the individual but that it probably has been modified in the species through caenogenesis.

Mechanism of magnesium polypnea in the urethanized cat. V. E. HALL AND W. J. WHALEN.* *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.*

Analysis of the mechanism whereby magnesium chloride given intravenously in small doses produces a polypnea resembling thermal panting has been pursued along two lines. First, in an effort to determine whether magnesium directly stimulates panting centers, or depresses regions restraining the panting centers, advantage was taken of the antagonism of magnesium narcosis by calcium salts. Calcium chloride solutions alone neither evoke nor alter panting in the urethanized cat. When the plasma magnesium concentration is elevated to 4–8 mg.% by magnesium chloride administration, the resultant polypnea is not affected by simultaneous administration of calcium chloride. When the plasma magnesium reaches 8–10 mg.%, respiration is slowed, but with simultaneous calcium administration polypnea is still present. Thus, calcium has no effect on magnesium polypnea but prevents respiratory depression by high magnesium levels. From this we conclude that magnesium polypnea results from direct stimulation of the panting mechanism, and not from narcosis of centers restraining the panting mechanism. Second, we have sought the anatomical site of magnesium action which evokes polypnea. A variety of intracranial procedures have been carried out. In general those preventing magnesium polypnea also abolished or markedly depressed thermal panting, a finding consistent with the view that heat and magnesium have a common site of action. More precise localization of the central action of magnesium is now being attempted by intracranial injection with the aid of a Horsley-Clarke stereotaximeter.

Hemodynamic responses during electronarcosis. W. F. HAMILTON, C. H. THIGPEN,* H. M. CADDELL* AND R. G. ELLISON.* *Depts. of Physiology and Psychiatry, Med. College of Georgia, Augusta.*

Alternating current of 175–250 ma. was administered using bitemperal pad electrodes. This current, about ⅓ of that usually used in electric shock therapy produced tonic and clonic seizures. The current was then reduced to 60–90 ma. and electronarcosis set in. Narcosis was maintained for about 10 minutes by small modulations of the current. The pressure pulses were recorded optically from the brachial artery during treatment. Some patients were undrugged and some were under the influence of either pentothal, curare, atropine, tetraethylammonium, or combinations of these drugs. After the current was turned on, the heart

was regular and the pressure mounted to such levels as 180/130 mm. Hg. As the pressure went up the heart became irregular. There were apparent periods of asystole lasting 8–10 seconds but the muscular spasm maintained a high pressure between pulsations. During the irregularity the pressure might rise to levels of 400/175. After the seizure the pressure declined to about 240/130 usually remaining hypertensive until the patient wakened. Pentothal, curare, and atropine had little effect on the pressure rise; atropine eliminated the cardiac irregularity. The fact that the pressure rise occurred after curare paralysis shows that it was due to vasoconstriction rather than to skeletal muscle spasms as in metrazol convulsions. TEA in doses of 240 mg. had little effect on resting pressure in most patients but aborted the hypertension and diminished the cardiac irregularity associated with electronarcosis. It is as effective as atropine in preventing buccal secretions.

Threshold and discrimination of differences in intensity of stimulus for deep pain. JAMES D. HARDY, HAROLD G. WOLFF AND HELEN GOODELL.* *Depts. of Physiology and Medicine, Cornell Univ. Med. College, New York City.*

Pain from the periosteum, galea and subcutaneous tissues of the forehead was elicited by pressure. The stimulator consisted of a plunger acting against a spring, the compression of which was measured in grams. The tip of the plunger which was pressed against the skin was a plastic hemisphere 1 cm. in diameter. To measure the pain threshold, the tip of the plunger was placed against the forehead of the supine subject and the pressure increased until the subject reported barely perceptible pain. The pain was of an aching quality. The difference in intensity of two stimuli evoking a just noticeable difference in deep pain sensation was measured in a stepwise fashion from the pain threshold to the stimulus level beyond which no further discrimination was possible. In 820 measurements of pain threshold by this method on 13 areas of the forehead of 3 subjects a force of 550 ± 60 grams was found to induce threshold pain. The just noticeable difference (jnd) in stimulus strength averaged 120 grams for stimuli between threshold and 1000 grams. For greater stimuli the jnd increased until at roughly 10,000 grams ability to discriminate seemed to be lost. The pain so provoked was comparable to the ceiling pain previously described for cutaneous pain. Twelve jnd's exist between threshold pain and the intensity of pain beyond which no discrimination was possible, as compared with 21 jnd's for cutaneous pain induced by thermal radiation.

Quinidine lactate and gluconate in delayed ventricular tachycardia following experimental coronary occlusion. A. SIDNEY HARRIS, ANTONIO ESTANDIA* AND REX F. TILLOTSON.* *Dept. of Physiology, Baylor Univ. College of Medicine, Houston, Tex.*

More than 40 dogs were used. Ventricular tachycardia developed 4½–8 hours after occluding the anterior descending artery as described previously. Drug tests began 15–20 hours after occlusion when the ventricular ectopic rate was between 180 and 250/minute. Each dose (after some preliminary experiments) was 10 mg/kg., given slowly by vein during a period of 5 minutes. Typically, the first dose of either quinidine lactate or gluconate reduced the ectopic rate to some degree, and in rare cases to zero. Such effects from a single dose were of brief duration, 10–30 minutes at most. Doses at intervals of one hour or longer produced temporary reductions only, ectopic rates returning approximately to preceding levels between doses. The optimally effective interval between doses was about 30 minutes. In a few dogs 2 or 3 doses at half-hour intervals reduced the ectopic rate to zero and maintained it at a low level for several hours. In other dogs with similar ectopic rates, a larger number of injections were required. Usually, higher ectopic rates were most difficult to control. Sufficient quinidine to control ectopic activity produced vomiting, diarrhea, and central nervous system reactions in some animals. These toxic reactions and arterial hypotensive effects were reduced by dilution and slow administration. Quinidine lactate was more effective and more toxic than gluconate. Gluconate produced sinus tachycardia more often. Morphine, 5 mg/kg., eliminated toxic reactions and reduced the amount of quinidine required.

Interspecific and intraspecific variation of lethal temperatures in fish. J. S. HART (introduced by RICHARD BERNARD). *Ontario Fisheries Research Lab., Univ. of Toronto, Toronto, Ontario, Canada.*

Lethal temperatures of fish compared after acclimation at a series of temperatures have shown large interspecific variations. These variations are manifest in both the pattern of time-temperature mortality relationships at temperatures that kill all of the fish, and also in the pattern of upper and lower tolerance levels at temperatures that kill 50% of the fish. The upper lethal temperatures are correlated with habitat. Species from cold lakes or streams generally have the lowest lethal temperatures, those from warm streams have intermediate lethal temperatures, while those from base level streams and marshes usually have the highest lethal temperatures. In contrast, intraspecific variation was small. Geographic variation of lethal temperatures was observed in 3 out of 9 species studied and this was large within only one species—*Micropterus salmoides*. It is possible that accessory factors may have complicated the picture for this species. The general similarity of

lethal temperatures among the geographic races of a given species is probably associated with the similarity of the habitats of these races.

Transformation of hexosephosphates by the cockroach. C. C. HASSETT. *Med. Div., Army Chemical Center, Md.*

The deposition of four hexosephosphates in insect muscle was studied by labeling them with radiophosphorus and measuring the activity of P^{32} as it accumulated in the coxa. It was found that passage from the digestive tract to the muscle was accomplished most rapidly by fructose diphosphate, followed by glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate. The presence of Zn in the gut inhibits the movement of the two latter compounds, indicating the presence of phosphoglucomutase in the gut.

Effect of antihistaminic agents on nystagmus. J. E. HAWKINS, JR. AND W. J. O'SHANNY.* *Merck Institute for Therapeutic Research, Rahway, N. J.*

The effect of certain antihistaminic agents on the vestibular and optokinetic components of nystagmus induced by rotation has been studied in the cat. Electronystagmograms show that none of the drugs tested affects the vestibular response directly. With Neo-Antergan, Phenergan and Pyribenzamine (2.5–20 mg/kg.), the primary change is in the optokinetic response, as shown by a reduction as great as 50% in the nystagmus during rotation with the eyes open, and by the emergence of a post-rotational nystagmus which is normally absent at speeds less than 30 rpm. This post-rotational nystagmus is interpreted as the result of a release of the vestibular centers from normal inhibition by the optokinetic mechanism during deceleration. It seems to be related to the mild sedative effect of the antihistaminics, which vary considerably in their rank order of CNS-depressant action from one species to another. Because of these species differences nystagmus studies in animals are not proposed for the selection of compounds for trial in the prophylaxis of motion sickness in man. Thus neither Benadryl nor Dramamine, which have been found effective in motion sickness, affects optokinetic nystagmus in the cat. Instead both elicit signs of CNS excitation with a strong autonomic component. Pentobarbital in small doses (5–10 mg/kg.) can depress optokinetic nystagmus as does Neo-Antergan, although in most experiments pentobarbital eliminates also the vestibular nystagmus. The action of Neo-Antergan and of pentobarbital can be readily overcome by amphetamine, which in the normal cat increases both the rate of nystagmus and the speed of the eye movements.

Contractile properties of compressed monolayers of actomyosin. TERU HAYASHI (introduced by RITA M. GUTTMAN). *Dept. of Zoology, Columbia Univ., New York City.*

The retention of certain biological properties of protein molecules physically altered at an air-water interface has been demonstrated in this laboratory for pepsin-albumin, and for catalase. The work was extended to a study of 'surface-denatured' actomyosin because of the possibility offered of relating the contractile process to intracellular structures. Actomyosin was extracted from rabbit muscle with Weber-Edsall solution, precipitated by dilution with distilled water, the precipitate then being dissolved in 0.6 M KCl to form a concentrated solution. This solution was then placed dropwise at the air-water interface of a Langmuir trough (buffered at pH 7.0 with diluted citrate-phosphate buffer) to form a monomolecular film. With compression of the film (Devaux effect) fibers of actomyosin were formed. Fibers thus formed differed markedly in appearance from actomyosin fibers formed by precipitation of the solution extruded through fine capillary tubing into distilled water, especially with regard to the presence of a longitudinal structural organization. The film-fibers, when treated with 0.3% ATP in 0.001 M $MgCl_2$ and 0.05 M KCl contracted optimally to 50% of the original length. The contraction appeared to be only along the long axis. Analyses of the water content of the fibers showed no marked difference between the uncontracted and contracted fibers. The simple technique of hanging weights on the fibers demonstrated that the fibers not only have considerable tensile strength, but under the influence of ATP, are capable of pulling a load through a distance; i.e. doing work.

Determination of residual volume of blood in right ventricle of normal and diseased human hearts. R. HEIMBECKER,* D. CARROLL,* W. FALHOLT,* G. MUDD,* C. FERENCZ* AND R. J. BING. *Dept. of Surgery, Johns Hopkins Univ. and Hospital, Baltimore, Md.*

A method has been described in a preceding communication which permits the estimation of the residual volume of the right ventricle of man (BING *et al. Am. J. Physiol.* 163: 698, 1950). The method consists in the insertion of a double lumen catheter into the right ventricle and the pulmonary artery respectively. Evans Blue is then injected into the right ventricle and the dye and blood mixture is withdrawn through a photoelectric dye recorder. From the slope of the dye dilution curve as plotted on semilogarithmic paper, the residual volume in the right ventricle may then be estimated. This method has now been applied to the determination of the residual volume in patients with normal and abnormal hearts. In addition, the systolic discharge, minute volume

and the ratio of residual volume/systolic discharge were obtained in every instance. In the normal man, the right ventricular residual volumes averaged 60 cc. and the RV/SD ratio was unity. In patients with right ventricular failure due to arteriosclerotic or hypertensive heart disease, the residual volumes averaged 260 cc. The ratio RV/SD was 9. In a series of patients with mitral valvular disease the right ventricular residual volume was incresaed (average 260 cc.). The ratio RV/SD was 9. However, in 5 of these patients the residual volumes were within normal range. It is probable that large residual volumes in mitral disease indicate the presence of myocardial failure. In patients with constrictive pericarditis the residual volume showed large variations. Digitalis caused a decrease in the residual volume of the majority of patients not suffering from congestive failure.

Effect of hypoxia and oxygen poisoning on pulmonary edema. ALLAN HEMINGWAY. *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

Pulmonary edema in guinea pigs has been measured quantitatively to determine lung weight, volume, and density and analyzing the extirpated lung for soluble (non-hemoglobin) protein, pulmonary hemoglobin, and insoluble protein nitrogen. Guinea pigs were placed in a closed system containing nitrogen and oxygen with the oxygen concentration being progressively lowered. At the instant of heart failure as indicated by the electrocardiogram the animals were removed, guillotined and the lungs analyzed as described above. There was no evidence of pulmonary edema as indicated by significant deviations from control values using the analytical procedure described. When guinea pigs were subjected to atmospheres of almost pure oxygen, pulmonary edema and vascular congestion occurred. There was little edema during the first 24–48 hours of the high oxygen environment. After this latent period edema progressively developed causing death in 5–6 days.

Certain hemodynamic factors concerned with control of blood volume. J. P. HENRY AND O. H. GAUER.* *Aero Med. Lab., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.*

Changes in effective blood volume appear to cause the kidneys to make compensatory adjustments in water and sodium excretion (PETERS, J. P. *New England J. Med.* 239: 361, 1948). Seven morphinized dogs have been submitted to hemorrhage and 2 of these to antecedent plasma infusion. In all cases arterial blood pressure and urine flow were continuously recorded by electrical transducers. Urine flow was halved following a hemorrhage of 20% of the estimated blood volume and doubled following a plasma infusion of 20% of the estimated blood volume. Yet the mean arterial pressure remained relatively undisturbed; indeed there was even a tendency towards overcompensation. On the other hand in 3 cases, serial x-rays of the heart showed a significant decrease in the heart size following hemorrhage. The data were inconclusive after infusion. Lung density decreased following hemorrhage. It is suggested that the changes in blood volume are detected by nervous receptors in the thoracic viscera, exclusive of the aortic arch receptors, (SCHWIEGK, H. *Arch. f. d. ges. Physiol.* 236: 206, 1935. SCHAEFER, H. *Ergebn. der Physiol.* 46: 71, 1950) and so induce the appropriate changes in the peripheral vascular bed and in renal function.

Comparison of physically active and inactive men 45 to 54 years of age. AUSTIN HENSCHEL. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

From 300 clinically normal men 45–54 years of age of similar social, economic and racial background, the 31 who had been physically most active and the 34 least active were selected. The physically active group had since early adulthood systematically participated in athletics, gymnasium work and recreational activities that frequently involved moderate to hard physical exertion while the inactive group had always been relatively sedentary. Comparisons were made on the basis of 56 separate test items. Data were obtained on blood pressure and pulse rates at rest, in work and in recovery, electrocardiographic and ballistocardiographic items, anthropometric measurements, response to the cold pressor test and blood hemoglobin and blood cholesterol concentrations. On only 4 of the items were the differences between the groups statistically significant. Three of the 4 items were anthropometric measurements. The active men were 5.4 kg. heavier, 6% larger around the chest and 2% larger around the abdomen. The extra weight was not fat; the body density was 1.046 and 1.040 in the active and inactive groups, respectively. In the active group the change in the diastolic blood pressure during work was greater. The differences between the 2 groups were small. The physical work involved in the usual types of recreational activities even when indulged in over many years appeared to have little measurable effect on physiological functions.

Effects of ultrasonic energy on growing bone. J. F. HERRICK, R. E. DEFOREST,* J. M. JANES* AND F. H. KRUSEN.* *Mayo Fndn. Rochester, Minn.*

In a recent publication we have reported that relatively high temperatures may be produced in the bone of experimental animals by ultrasonic energy. Other investigators have reported similar observations. We found that a rise in temperature of aproximately 10°C. can be produced by a 1.5-

minute exposure to 10 watts of ultrasonic power. The muscle, through which the ultrasound was propagated before reaching the bone, rose only 2°. These temperatures were observed 15 seconds after turning off the ultrasonic generator. This apparent selective absorption of ultrasonic energy by bone prompted us to study the effect of ultrasound on growing bone. Dogs ranging in age from 4–6 months were used in this particular study. The right hind leg served as a control. The left hind leg was shaved and ultrasonic energy was directed toward the medial surface of the upper tibial epiphyseal area. Water, degassed by boiling, was used as a coupling medium between the sound head and the animal's leg. Prior to each experiment the dog was anesthetized with Thiopental (Pentothal-sodium) administered intravenously. The effects of the ultrasound were observed by means of roentgenograms taken at various intervals before and after exposure for different periods with different outputs of the ultrasonic generator. The frequency of the ultrasonic vibrations was 800,000 cycles per second. It is concluded from the roentgenographic observations that ultrasonic energy is very destructive to the growing bones of the dog under the conditions of our experimental procedure.

Effects of ascorbic acid on thyrotrophin (TSH)-treated guinea pigs. ELIZABETH A. HERROLD,* ROY K. RINEHART,* HAROLD E. COOMBS* AND JAMES H. LEATHEM. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Immature male guinea pigs treated with TSH (Parke-Davis), one chick U/day, for 20 days, showed increases in oxygen consumption of 39, 45, 36 and 16% over controls at 5, 10, 15 and 20 days, respectively. When TSH-treated guinea pigs were given ascorbic acid, 25 mg/day by stomach tube, the increases in oxygen consumption were 43, 56, 44 and 34% over controls. Ascorbic acid administered to normal guinea pigs had no significant effect on oxygen consumption. When ascorbic acid was administered on *days 11–20* to pigs on TSH treatment for 20 days, the oxygen consumption was 29 and 25% above controls on *days 15* and *20*. TSH treatment clearly increased adrenal and thyroid weights and had a tendency to increase testis and seminal vesicle weights. Ascorbic acid did not alter the effects of TSH on organ weights except to enhance the increase in seminal vesicle weight. Studies of the oxygen consumption of animals with TSH, ascorbic acid, and some antithyroid compounds administered concomitantly are now in progress.

Head-turning and eye deviation evoked by cortical excitation in the freely moving cat. WALTER R. HESS,* DONALD A. MCDONALD* AND ROBERT B. LIVINGSTON. *Physiologischen Inst. der Univ., Zürich, Switzerland.*

A series of experiments were undertaken to analyze and to compare responses elicitable from the prefrontal cortex of the cat with those which can be evoked by stimulation of the diencephalon. (For technics see W. R. HESS, *Die Methodik der lokalisierten Reizung u. Ausschaltung subkortikaler Hirnabschnitte.* Leipzig: Thieme, 1932, 119 pp.; *Das Zwischenhirn, Syndrome, Lokalisationen, Funktionen.* Basel: Schwabe, 1949, 187 pp.) One of the cortical regions selected for particular study was the cingulate gyrus where in 6 animals we confirmed and elaborated earlier work by Hess on this same region (*Helv. Physiol. Pharmacol. Acta* 6: 731, 1948.) Complex visceral as well as somatic motor responses were evoked by excitation of this zone in the lightly anesthetized cat. In the freely moving, wide-awake cat, somatic responses included an elaborate pattern of controversive head and eye movements which had a long latency, sometimes as much as 30–45 seconds at intensities of 2–3 v. Impulses delivered at 2, 4, and 8 c/s initiated responses which were in phase with the rhythm of stimulation. Prolonged excitation resulted in head-turning followed by postural adjustments permitting a complete revolution of the animal's position on the table. Stimulation of some points evoked an upward or upward and backward head movement accompanying the lateral deviation, in which case the animal could be caused to rise up spirally or even topple over backwards by prolonged stimulation. If the head was prevented from turning, the eyes alone deviated. This response was accompanied by widening of the palpebral fissure, dilatation of the pupils, full retraction of the nictitating membranes and rhythmic conjugate deviation of the eyes toward the side opposite to the hemisphere stimulated. On discontinuation of excitation, the cat recovered its normal posture, composure and general behavior as though nothing unusual had happened. Stimuli could be repeated indefinitely with remarkable uniformity of the total performance as long as the stimulus parameters and the intervals between successive periods of stimulation were kept constant.

Rapidly acquired tolerance to decompression in insects. WILLIAM A. HIESTAND AND FRED W. STEMLER.* *Lab. of Animal Physiology, Purdue Univ., Lafayette, Ind.*

Tolerance to decompression was observed in insects as a result of previous exposure(s) to extremely low (0–1 mm. Hg) barometric pressure. Various insects were placed individually in decompression chambers consisting of the round end of test tubes cut off at appropriate lengths. By means of a vacuum pump the insect was ex-

plosively decompressed and survival measured to the nearest second judged by the last visible movement of an appendage. The insect was then kept at the low pressure for 3 minutes after which it was returned to room pressure and allowed to recover. Repeated decompressions were carried out at 10-minute intervals. Insects showing 'acclimatization' to repeated decompression included *Diptera* (*Syrpha, Culex, Calliphora, Musca*); *Hymenoptera* (*Polistes*); *Coleoptera* (*Coccinella, Anthrenus*); *Hemiptera* (*Reduviis*). The house fly is typical of the results obtained. For example, the average survival in seconds with decompressions spaced at 10-minute intervals was as follows: 7.8, 27.7, 35.8. After 30 minutes, survival remained fairly constant up to about an hour and then began to decrease. Recovery after each decompression occurred in 3 to 5 minutes. Tolerance to decompression was still retained for as long as 40 hours. Flies exposed first to nitrogen were more tolerant to decompression as well as those exposed first to decompression being more tolerant to nitrogen anoxia. The greatest acquired tolerance was shown by the mosquito, *Culex*, the least by the ladybird, *Coccinella*. Isolated legs of *Musca* and *Tenebrio* when decompressed or exposed to nitrogen for 3–5 minutes occasionally showed slight movement.

Relation of ambient water temperature to survival to drowning and influence of ethyl alcohol and epinephrine on survival. WILLIAM A. HIESTAND, FRED W. STEMLER,* AND JACOB E. WIEBERS.* *Lab. of Animal Physiology, Purdue Univ., Lafayette, Ind.*

Survival to drowning in mice (Purdue Swiss strain) is inversely proportional to the temperature of the surrounding water. Thus mice survive 67.4 seconds when submerged in water of 4°C.; 61.9 seconds at 25°C.; and 44.8 seconds at 45°C. Ethyl alcohol injected intraperitoneally (10 ml/kg.) 10 minutes before immersion prolonged survival at 25° C. to 94.8 seconds (an increase of 53%); and increased survival of 45° C. to 64.4 seconds (an increase of 44%). Injection of epinephrine (1 mg/kg.) intraperitoneally 10 minutes before immersion shortened survival time to 52.2 seconds (a decrease of 16%). Both sexes were used and females were found to survive drowning 3 to 13% longer than males. Strain differences in survival to drowning exist. Mice of an unknown Swiss strain survived for an average of 52.8 seconds at 45° C. These findings agree with previous experiments in our laboratory on alcohol prolongation of survival to anoxic death by explosive decompression but disagree with the effects of epinephrine on decompression survival. These results agree in principle with those of Gray who used rats (GRAY, S. W. *Anat. Rec.* 108:(3) 130, 1950).

Serum calcium and inorganic phosphorus levels following calcium injections into nephrectomized, bilateral ureteral ligated and thyroparathyroidectomized dogs. JAMES R. HINES* AND SMITH FREEMAN. *Dept. of Exp. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

A comparison was made of the rate of removal of intravenously injected calcium (15 mg. Ca/kg.) before and after nephrectomy, ureteral ligation, and thyroparathyroidectomy with subsequent nephrectomy. The rate of disappearance of calcium was uniformly slower than normal in 8 dogs 48 and 96 hours after bilateral nephrectomy. In normal animals the serum inorganic phosphorus rose after calcium injection; after nephrectomy this rise was more pronounced. The average of 8 dogs 48 hours after ureteral ligation showed that the calcium disappeared more rapidly than seen in the controls. In this group the average inorganic phosphorus levels did not go up following calcium injection as in the normal and nephrectomized dogs. Eight dogs that were thyroparathyroidectomized showed a slight delay in calcium removal, both in the hypocalcemic state and after the serum calcium had returned to normal under vitamin D therapy. The inorganic phosphorus increase following calcium injection was similar to their control values. After nephrectomy, the calcium disappearance curves, as well as the inorganic phosphorus response to calcium injection closely resembled that of the nephrectomized normal animals. However, the rate of increase in N.P.N., serum potassium and inorganic phosphorus was slower, and the survival time longer, in the thyroparathyroidectomized-nephrectomized animals than following nephrectomy alone. The results obtained in this study indicate that factors other than the concentration of inorganic phosphorus regulate the disappearance of calcium from the blood of dogs deprived of their renal excretory function. In addition, the serum inorganic phosphorus response to injected calcium is independent of the thyroparathyroid apparatus, but is influenced by the presence of the kidney.

Oxygen consumption of airline pilots during regular scheduled flights. FRED A. HITCHCOCK. *Med. Research Lab., Civil Aeronautics Administration Aeronautical Center, Oklahoma City, Oklahoma.*

The respiratory metabolism and energy output of airline pilots while flying twin engine aircraft (DC-3) on regular scheduled runs has been measured by means of the Douglas bag Haldane technic. Eastern Airlines flights 451 (westward by day) and 454 (eastward by night) between New York City and St. Louis were used in this investigation. Tests were run on the pilots before the flight and after its completion, and during straight and level

flying between New York City and Washington, D. C., between Washington and Charleston, W. Va., while the plane was at maximal altitude (about 8,000 ft.) and between Evansville, Ind. and St. Louis. Four tests on 3 pilots were made during takeoffs and landings. In all 33 flights (17 by day and 16 by night) using 13 different pilots were made. The pilots ranged in age from 31 to 38 years. The average oxygen consumption for all tests run during night flying was 341 cc/minute and the corresponding figure for day flight was 337 cc/minute. With both day and night flights the highest figure was obtained on the first leg of the flight, while the lowest figure was obtained while flying over the mountains at an average altitude of 8,000 ft. For takeoffs and landings the average figure was 407 cc/minute. Because the resting tests run before and after night flights were lower than those obtained for day flights night flying caused an increase in oxygen consumption of 26% and day flying of only 15%.

Nature of the hibernation of bats. RAYMOND J. HOCK (introduced by LAURENCE IRVING). *Arctic Health Research Center, U. S. Public Health Service, FSA, Anchorage, Alaska.*

Bats are among the hibernating mammals (the heterotherms), and in general exhibit the phenomena of that condition. A study of their body temperatures and oxygen consumption in relation to environmental temperatures reveals that in some important respects they are unique. From 2° to 37°C., body temperature closely and rapidly parallels environmental temperature. Log oxygen consumption plotted against temperature over this range is nearly a straight line. From these data it appears that bats do not possess thermoregulatory mechanisms like those of other mammals. Other hibernators cannot be examined at many body temperatures, for they maintain a rather constant high temperature when not hibernating, a more or less constant low temperature during hibernation, and rapidly changing temperature when emerging from or passing into hibernation. It thus appears that no other mammals are known in which metabolism can be accurately referred to body temperatures over a wide range. Furthermore, the body temperature of bats quickly falls to that of their environment every time the bat comes to rest. Unlike other heterotherms they do not require a prolonged and complex sequence of events to prepare for hibernation. From the literature it appears that the tissues of hibernators differ from those of homoiotherms in their ability to withstand long periods of low temperature. The tissues of bats are similar, but the organization of the animal seems to be different. A metabolic classification of air-breathing vertebrates might be: homoiotherms, heterotherms, bats, and poikilotherms. Bats differ from the last in that they are able, through control of their muscles, to quickly raise their body temperature above that of the environment.

Disorders of renal function produced by cerebral stimulation. E. C. HOFF AND J. F. KELL, JR.* *Neurological Science Lab., Med. College of Virginia, Richmond.*

Hoff *et al.* (*Federation Proc.* 8: 76, 1949) reported that electrical stimulation of foci on gyrus proreus, gyrus sigmoideus, and orbital and mesial surfaces of the cat's cerebral cortex evoked transient elevations of blood pressure associated with a pronounced vasoconstriction in the cortex of both kidneys. This renal cortical ischemia has been demonstrated by direct visualization of the kidney under white light; by the injection of fluorescein solution and observing the kidney under ultra violet light; by intra-arterial injection of an India ink solution at the height of the pressor response; and finally by the evidence of widespread fatty degeneration of the renal tubular cells. Following transection of the cervical spinal cord or intravenous administration of Dibenamine, cerebral cortical stimulation no longer produced any rise in blood pressure or renal ischemia. Renal cortical ischemia and tubular degeneration were more severe following Brief Stimulus Therapy Apparatus (BST) stimulation with the electrodes spanning the cat's cerebral pressor areas. In chronic experiments with BST stimulation, the kidneys revealed a picture of lower nephron nephrosis. With chronic BST stimulation alone the renal lesion has always been found to be reversible. In chronic experiments in which BST stimulation was followed immediately by withdrawal of sufficient blood to produce mild or moderate shock (about 15% of total blood volume), cats recovered and showed usual activity for a week to 10 days and then developed spontaneous convulsions and extensor rigidity and died 5–7 days later with severe lower nephron nephrosis.

'Vulnerable' period and cardiac excitability curve. BRIAN F. HOFFMAN,* EDWARD F. GORIN,* FLORENCE S. WAX,* EUSTACE E. SUCKLING,* ARTHUR A. SIEBENS* AND CHANDLER MCC. BROOKS. *State Univ. Med. Center, New York City.*

Excitability curves were obtained from the turtle's ventricle and from the auricle and ventricle of the cat by determining the threshold of excitability at various intervals of the cardiac cycle. Test shocks of certain fixed durations (3, 5 or 10 milliseconds) were used in plotting each curve. Vulnerability to fibrillation was also determined by increasing the intensity and duration of the test shocks applied at the various intervals. As in the dog (*Am. J. Physiol.* 163: 272, 1950;

162: 219, 1950) 'dips' occur in the excitability recovery curve during the relative refractory period. It was found, as in the case of the dog's auricle (*Am. J. Physiol.* 1951, in press), that, during this interval of the cycle showing the unsustained recovery or dip phenomenon, the auricle and ventricle of the cat and the ventricle of the turtle were peculiarly vulnerable. Supra-threshold test shocks produced multiple responses or actual fibrillation when applied at this time but elicited only single extra systoles elsewhere in the cycle.

Thirst with thyroid feeding. J. H. Holmes and M. Behan. *Dept. of Medicine, Univ. of Colorado School of Medicine, Denver.*

As part of our study of thirst, observations have been made on the polydipsia induced by thyroid feeding to determine if any consistent fluid changes appeared which might be correlated at least in part with the mechanism of thirst. Five dogs were studied over periods ranging from 3–6 months with thyroid dosage ranging from 16–80 grains per day. Measurements were made at frequent intervals of plasma volume (T1824), extracellular volume (NaSCN), the hematocrit value, serum concentrations of protein, chloride and sodium and urinary volumes, specific gravity and chloride concentration. The fluid intake increased in every instance on thyroid feeding, the maximum increase amounting to five to eight times that of the control periods. In most instances, the drinking was greater with .9% NaCl, amounting to 1000–2000 cc. more per week than with water. There was a corresponding increase in urine output. There did appear to be a consistent increase in extracellular fluid (NaSCN) in three instances, the maximum as much as 1000 cc. In two instances with the maximum dose of thyroid there was an associated increase in the serum sodium concentration. This did not correlate in any way with the increase in water intake. There were no consistent changes in plasma volume. In two instances there were slight decreases in the hematocrit value and the plasma protein concentration. Where the hematocrit value dropped, there was a corresponding decrease in the calculated blood volume. There were no fluid balance changes in this study that could consistently. be correlated with the mechanism of thirst.

Physiological changes induced by high fluid intakes. J. H. Holmes and A. V. Montgomery.* *Depts. of Medicine and Physiology, Univ. of Colorado School of Medicine, Denver.*

Some years ago, several German authors stated that when fluids were forced in man for a period of time one could induce a prolonged and increased desire for water. In order to investigate this problem further, a device was designed to administer water to dogs by gastric fistula continuously throughout a 24-hour day for periods up to 14 days or more in duration. This method does not interfere with rest or free movement about the cage. The amounts of fluid given per day varied from approximately 8–14 liters. During the period of forced fluids the urine volumes approximated the intakes with the urine specific gravity ranging from 1001–1005. The urine chlorides dropped to values of 5–10 mEq/liter. Despite the low chloride concentration this may result in a considerable daily chloride loss when the urine volume approaches 12–14 liters per 24 hours. This procedure, therefore, frequently produced lowered serum chloride concentrations (90 mEq/liter) and a rise in blood pH to maximum values of 7.65 (control 7.45). On intakes less than 14 liters there was no evidence of significant fluid retention as shown by absence of weight increase or change in serum protein concentration or the hematocrit value. When the daily intake exceeded 10–14 liters of water intoxication could be induced. Observations on whether such a forcing of fluids can induce a persistent polydipsia are not at the present conclusive. For example, 1 animal who has had 4 periods of forcing of fluids now has a daily intake averaging over 3 liters as compared with a control of less than 1 liter.

Lack of evidence of a Trueta shunt in dogs during electrical stimulation of renal artery nerves. C. Riley Houck. *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

Physiologic evidence for shunting of blood from renal cortex to medulla, 'Trueta shunt,' during electrical stimulation of the left renal artery nerves was sought in 14 dogs using clearance, tubular saturation, and extraction techniques. The right kidney was not stimulated (control). Source of stimulation was a sinusoidal wave alternating current stimulator delivering from 4–14 v. at 2–4 cps, or a Harvard inductorium delivering from 10–25 v. at 30 impulses/sec. Morphologic evidence was sought by injecting during stimulation 50 cc. India ink through a catheter inserted via common carotid artery into the dorsal aorta above the branching of the renal arteries. In the stimulated kidney the following were consistently reduced: filtration rate, urine flow, blood flow, Tm_{PAH}, $Tm_{glucose}$, extraction percentages of creatinine and PAH, filtration rate per U of excretory tissue (filtration/Tm_{PAH}) and perfusion of active tubules with blood (RBF/Tm_{PAH}). The extraction percentage of oxygen and filtration rate per U of reabsorptive tissue (filtration/$Tm_{glucose}$) were consistently increased. Little or no change occurred in right kidney function. Injected ink was uniformly distributed throughout the control kid-

ney. In the stimulated kidney regions of ischemia (infarcts) involving both cortex and medulla were apparent, alongside regions heavily engorged with ink. Consequently, renal artery nerve stimulation failed to produce shunting of blood from cortex to medulla but rather random closing-off of nephrons, frequently localized and concentrated enough to be visible as infarcts. (Supported by a grant from the American Heart Association.)

Comparison of single injection and infusion methods for determining renal clearances in dogs without urine analysis. C. RILEY HOUCK. *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

Glomerular filtration rate (mannitol clearance) and renal plasma flow (PAH clearance) were determined in 10 Nembutalized female dogs each consecutively by *1*) the single injection method of Newman, Bordley, and Winternitz—C_{SS}—(*Bull. Johns Hopkins Hosp.* 75: 253, 1944), *2*) the infusion method of Earle and Berliner—C_{INF}—(*Proc. Soc. Exper. Biol. & Med.* 62: 262, 1946) both without urine analysis, and *3*) the infusion method suggested by Gaudino and Levitt—C_{SI}—(*Am. J. Physiol.* 157: 387, 1949). All values were compared with simultaneously determined urine clearances —C_U. For filtration rate, C_{SS}/C_U averaged 1.09; range, .96–1.29. C_{INF}/C_U averaged 1.16; range, .68–1.84. C_{SI}/C_U averaged 1.01; range, .85–1.40. In 7 of 10 dogs, agreement with C_U was within 10% for C_{SS}; 20% for C_{SI} and C_{INF}. In 3 dogs, agreement was 23%, C_{SS}; 48, C_{SI}; and 26, C_{INF}. For renal plasma flow, C_{SS}/C_U averaged 0.99; range, 0.80–1.78. C_{INF}/C_U averaged 1.13; range .90–1.64. C_{SI}/C_U averaged 0.89; range 0.71–1.07. In 3 of 10 dogs, C_{SS} agreed with C_U within 10%, while C_{SI} and C_{INF} agreed no better with C_U. In 2 of the 7 dogs, C_{SI} and C_{INF} agreed more closely with C_U than did C_{SS}; in 2, C_{SS} more closely than did the other two methods. The infusion methods appear no better than the single injection method which more frequently gives closer checks with the conventional urine method. (Supported by a grant from the National Institutes of Health, U. S. Public Health Service.)

Facilitation of mephanesin (lissephen) depressed stretch reflexes. RICHARD B. HOVDA (introduced by John S. GRAY). *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

At levels of mephanesin which suppress almost completely the static component of the stretch reflex from the gastrocnemius muscle of unanesthetized chronic spinal dogs and anesthetized intact dogs, the reflex may be caused to reappear by stretch of the contralateral gastrocnemius muscle or stimulation of the nerves from this muscle. Stimulation of contralateral cutaneous nerves, stretching of other muscles of the opposite extremity and nociceptive stimuli applied to either limb are ineffective in facilitating the depressed response. Successive, brief stretch of the ipsilateral gastrocnemius may result in an inconstant and slight increase in response. The facilitation occurs in the absence of any electromyographic evidence of crossed reflexes. The degree of facilitation is dependent upon the duration of stretch with an optimum of about 15 seconds. The period of facilitation may last one to two minutes after cessation of the contralateral stimulation. This facilitation does not occur when the myotatic reflex has been completely abolished by mephanesin, nor does it occur when the stretch reflex has been comparably depressed by either deep ether or sodium pentobarbital (Nembutal) anesthesia.

Effect of Terramycin on acute radiation syndrome in dogs. J. W. HOWLAND, F. W. FURTH,* AND M. P. COULTER.* *Atomic Energy Project, Univ. of Rochester, Rochester, N. Y.*

During the course of investigations on the role of infection in the acute radiation syndrome in dogs various antibiotics have been administered to dogs which have recived large doses of x-radiation. Previous investigations showed that Aureomycin reduced the morbidity and mortality of the acute radiation syndrome in dogs. Terramycin has a similar effect. Twenty-seven adult mongrel dogs were exposed to a single dose of 450 r of x-radiation from a 250 kv source. Immediately after irradiation 14 of the animals were started on a dose of approximately 100 mg/kg/24 hours of Terramycin administered in divided doses every 6 hours for 28 days. The remaining 13 dogs served as controls. By the 60th day post-radiation 12 (92%) of the control dogs had died whereas 7 (50%) of the treated dogs had died. A delay of 4–5 days in the onset of mortality in the treated group was found. The dogs in the treated group did not appear as sick as those in the control group. Blood cultures on both groups of dogs showed no significant difference in the incidence of positive cultures between the groups. No significant difference in the decrease in peripheral blood elements or in weight loss was noted in the two groups. (Based on work performed under contract with the United States Atomic Energy Commission at The University of Rochester Atomic Energy Project, Rochester, N. Y.)

Influence of some inorganic cations on the electrolyte pump in isolated frog skin. ERNST G. HUF AND JOYCE WILLS.* *Dept. of Physiology, Med. College of Virginia, Richmond.*

Net salt (NaCl) and water accumulation at the chorion layer of frog skin which has isosmotic salt solution (47 μeq/ml NaCl) on both sides was

studied in relation to absence or presence of the chlorides of either K^+, Rb^+, Cs^+ (1 μEq/ml) or Ca^{++} (2 μEq/ml). The solutions contained also 2 μEq/ml $NaHCO_3$. The pH was set at 7.5. Skin bags with the epithelial layer turned inward were filled with 5 ml. and immersed for 12 hours in 2 liters of salt solution at room temperature. Experimental bag and control bag came from the same frog. Changes in volume and in ion concentration of the fluid in the bag were measured. From these data the net transfer of ions was calculated. Omission of Ca^{++} from the salt solution did not change salt accumulation. Omission of K^+, however, lowered salt accumulation to about 60% of the control value, obtained from experiments with salt solutions containing K^+ (and Ca^{++}). H_2O transfer from the epithelial to the chorion layer was also somewhat reduced in the absence of K^+. Rb^+ almost completely and Cs^+ partially could substitute for K^+. Relative effectiveness of K^+: Rb^+: Cs^+ = 1:0.98: 0.76, with a value of 0.64 in case where none of the 3 cations were added to the salt solution. H^+, K^+, Rb^+ and Ca^{++} were accumulated at the epithelial layer. This was even found when K^+ or Ca^{++} were omitted from the solutions which suggests that K^+ and Ca^{++} of the skin were extruded. It suggests also that salt accumulation was partially maintained by intracellular K^+, if K^+ was omitted from the solutions.

Mechanism of reversal of spinal and bulbar reflexes. R. A. HUGHES,* G. W. STAVRAKY AND R. D. TEASDALL.* *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.*

Reflex responses in a limb vary when currents of different intensity are applied to the afferent nerve (Frohlich; Sherrington and Sowton; Hinsey, Ranson and Doles). Reflex reversal in both quadriceps muscles brought about by varying the intensity of afferent stimulation was studied in 16 previously intact spinal and decerebrate cats, and in 12 animals sensitized by an aseptic semidecerebration of 4 to 6 weeks' standing. Ipsilateral, then bilateral contraction of the quadriceps muscles occurred in spinal preparations, while a sequence of ipsilateral, bilateral, then crossed contraction and ipsilateral relaxation took place in decerebrate animals with an increasing intensity of afferent stimulation. Sensitization of corresponding spinal centers by preceding semidecerebration greatly predisposed these centers to the occurrence of reflex reversals. Stimulation of the peroneopopliteal nerve on the sensitized side resulted in ipsilateral inhibition, while stimulation on the opposite side led to a relaxation of both the quadriceps muscles, this taking place in spinal as well as in decerebrate preparations. The study was extended to the effect of vagal stimulation on the respiratory center with results corresponding to those of Wyss. Reversal from excitation to inhibition which occurs with increasing intensities of afferent stimulation is regarded as being due to 'overexcitation' of central neurones and not dependent solely upon stimulation of fibers of different thresholds in the afferent nerve. The greater tendency to reversals seen in previously denervated spinal centers is attributed to the greater ease with which supersensitive neurones may become overexcited as compared to normal neurones.

Respiratory response of anesthetized dogs subjected to progressive anemic hypoxia. WAYLAND E. HULL (introduced by J. W. WILSON). *Dept. of Physiology, Duke Univ. School of Medicine, Durham, N. C.*

Using a wire screen tracheal airflow meter, registering optically by means of sensitive differential manometer and photokymograph, respiratory responses were recorded on anesthetized dogs subjected *a*) to progressive hemodilution by the process of controlled blood loss and simultaneous infusion of plasma or plasma substitute, and *b*) to progressive hypoxia resulting from inhalation of air containing 0.6% to 1.0% carbon monoxide by volume. The respiratory responses to decreased oxygen capacity are similar for the condition of simple anemia in which the amount of circulating hemoglobin is decreased, and in the condition where the normal amount of hemoglobin is present, but non-functional by its combination with carbon monoxide. Depression of tidal volume is characteristic, the initial stimulant phase being of short duration or absent. Breathing rate is increased, decreasing only at the point of respiratory collapse. Sudden administration of oxygen results in increased respiratory activity, an increase in tidal volume, often with a slight decrease in breathing rate. The pattern of air flow of breathing may be accurately interpreted as reflecting the state of respiratory neuromuscular activity. The technique of controlled blood loss and infusion of plasma is a useful physiological tool for the study of the hypoxia progressive simple hemodilution while maintaining normal blood pressure to the point of respiratory failure.

Effect of cold on joints. J. HUNTER AND M. G. WHILLANS.* *Defence Research Med. Labs., Toronto, Canada.*

On exposure to cold 'stiffness' in joint movement results in a loss in manual dexterity. Of the factors responsible for joint stiffness it has not been clear how much is a result of a local physical change in the state of tissues making the joints more resistant to movement, how much is due to increased muscle tone or spasm accompanying the pain or whether it is a combination of these

factors. Exposure to zero and subzero ambient temperatures results in a significant fall in joint temperature where the knee joint of the cat was used as test object. Body, muscle and *average* skin temperatures were also recorded. The fall in these values was of lesser degree than the fall in joint temperature. The fall in joint temperature is associated with an increase in friction of the joint freed from muscular attachments with exception of cruciate ligaments. Under the conditions of the experiments the effects of possible changes in excitability of the central or peripheral nervous systems were minimal because the moving force was applied directly. The possible effect of muscle tensions was eliminated by severing the muscles bearing over the knee. A physiologically important component of joint stiffness resulting from exposure to cold must, therefore, result from a local physical change in the joint tissues.

Estimation of adrenaline-*nor*adrenaline solutions by quantitative paper chromatography. D. E. HUTCHEON (introduced by L. B. JAQUES). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

The chief difficulty in the assay of adrenaline and *nor*adrenaline by paper chromatography has been to obtain well-defined areas on the filter paper. Oxidation of the amines on the chromatogram to the adrenochrome or iodoadrenochrome derivatives results in spots with indistinct margins that are difficult to measure. However, when the paper is developed in osmic acid vapour, the osmic acid is reduced by these amines and black, discrete areas appear for both adrenaline and *nor*adrenaline. The area of these spots is proportional to the log of the amount of amine applied so that the concentration of adrenaline and *nor*adrenaline in an unknown solution or extract can be determined by applying it to filter paper, making the chromatogram, and comparing the areas with those obtained by the application of known mixtures. By quantitative paper chromatography, rat suprarenals were estimated to contain 0.79 μg. adrenaline and 0.19 μg. *nor*adrenaline per mg. gland.

Effect of caffeine upon motor activity of human stomach. EDOUARD JACOBS* AND JAMES L. A. ROTH. *Dept. of Gastroenterology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

The effect of caffeine upon gastric motility was evaluated by *1*) periodic fluoroscopic observations of a barium meal and by *2*) balloon-kymograph recording of antrum motor activity. Fifteen healthy, asymptomatic young adults served as subjects for at least three 'blind' observations. Each subject served as his own control for placebo studies. The barium meal (100 gm. barium sulfate, distilled water to 300 cc.) was administered 20 minutes after a placebo (250 mg. sodium benzoate in 30 cc. water) or after caffeine sodium benzoate (500 mg. in 30 cc. water). Gastric emptying was observed fluoroscopically every 15 to 30 minutes until only a trace of barium remained in the stomach. Tracings were made of the fluoroscopic screen for permanent record of the relative changes in surface profile. The mean emptying time after the placebo was 85 minutes and after caffeine, 77 minutes. The surface profiles revealed 29.2% of the barium remained in the stomach 45 minutes after the placebo and 24.6%, after caffeine. There was a slight tendency for caffeine to accelerate gastric emptying, but these differences were not statistically significant. The intravenous injection of 250 mg. caffeine sodium benzoate produced no definite influence upon the interdigestive activity of the gastric antrum recorded by a small balloon located under fluoroscopic guidance and inflated with 30 cc. of air. These experiments exclude any significant influences of caffeine (in usual dose) upon gastric motility in normal human subjects. The possibility of augmentating gastric motility in subjects with excessive vagus nerve activity has not been excluded.

Protection against diabetes induced by alloxan. RALPH G. JANES. *Dept. of Anatomy, State Univ. of Iowa, Iowa City.*

Several chemical compounds, some unrelated, have been used in an effort to block the idabetogenic action of alloxan. Two hundred and fifty-eight adult male rats of the Long-Evans strain were used, 48 of which received only alloxan (115 mg/kg.). The remaining animals received the test compounds intraperitoneally and was followed by subcutaneous injections of alloxan. Statistically significant protection ($P < 0.001$ by the X^2 method) against alloxan diabetes was found when the following chemicals were used: niacinamide, benzamide, N_1N-dicyclohexyl benzamide dissolved in propylene glycol, N-cyclohexyl benzamide dissolved in propylene glycol, 1 N ammonium hydroxide, and propylene glycol. Some protection was afforded apparently by niacin, cinchophen dissolved in 1 N ammonium hydroxide, ethyl alcohol, and sodium lactate, but the statistical significance of the findings of these groups could not be established unequivocally. No protection was found with acetamide or urea. Because of the large number of diversified chemical compounds which have been described that block the action of alloxan, it is difficult to establish what mechanism may be involved in this protection. In the present study, the diabetogenic properties of alloxan were probably not destroyed at the site of injection because the chemical materials which might nullify its action were administered in a different region of the body.

Some properties of cell-free gastric mucus. HENRY D. JANOWITZ* AND FRANKLIN HOLLANDER. *Gastroenterology Research Lab., Mount Sinai Hospital, New York City.*

By stimulating the mucosa of canine fundic pouches topically with acetylcholine or related parasympathomimetic drugs, we have obtained viscous alkaline mucus, free of columnar cells, in quantities suitable for study of its properties. Of these, the viscosity was measured by the Ostwald technique at 37°C. Under standardized conditions of stimulation the relative viscosity ranged widely from experiment to experiment (29–260). Serial dilution curves had similar shapes, but no simple relationship between concentration and viscosity was evident. Lowering of pH 5.2 led to a reversible increase in viscosity; below this coagulation occurred. Native mucus loses its high viscosity on incubation at body temperature, approaching the fluidity of water after 24 hours. This 'spontaneous' loss of viscosity is accelerated by addition of cysteine, and retarded by oxalate or citrate (removing calcium ions). Native mucus stimulated by acetylcholine was rapidly liquified by proteolytic enzymes: trypsin, KCN-activated papain, at pH 7.0; pectinase at pH 5.5. Lysozyme, hyaluronidase, snail mucinase, and human salivary amylase had no such effect. This mucus contained considerable amounts of pepsinogen, and a small but constant amount of lysozyme. No protease was demonstrated using hemoglobin substrate at pH 7.0. These findings suggest that (1) protein linkages of gastric mucin are important for its high viscosity and (2) under physiological conditions there is a continuous slow process of liquification of the surface layer of gastric mucus. (Aided by a U. S. Public Health Service grant.)

Relation of glomerular filtration rate and blood and urine pH to physiologic proteinuria. NORMAN B. JAVITT (by introduction) AND A. T. MILLER, JR. *Dept. of Physiology, Univ. of North Carolina Med. School, Chapel Hill.*

Glomerular filtration rate, blood and urine pH and urine protein were measured in human subjects performing exhausting exercise. In some experiments the subjects were given sodium bicarbonate or ammonium chloride one hour before exercise. Proteinuria invariably resulted when the glomerular filtration rate was diminished by 40% of the control value and total urine protein varied directly with the depression of filtration rate. There was no significant alteration in urine pH and no protein in the urine voided immediately after exercise. In urine samples obtained 5–10 minutes post-exercise, pH was depressed 1–2 units and proteinuria was maximal. By 20 minutes post-exercise, protein had disappeared from the urine, but urine pH was still maximally depressed, which argues against a direct causal relation between urine acidity and proteinuria. Pre-exercise alkalinization decreased the post-exercise fall in urine pH and diminished the proteinuria, while the reverse was true of pre-exercise acidification. In neither case was the magnitude of the depression of filtration rate (and hence presumably the degree of glomerular membrane anoxic damage) affected. It is suggested that acidification of the renal parenchyma by renal vasoconstriction, as demonstrated by Olsen and Schroeder (*Am. J. Physiol.* 163: 181, 1950) and by exercise lactacidemia is an important factor in the causation of exercise proteinuria.

Pressure and flow pulses in carotid artery of the dog compared with voltage and current pulses in electrical analogs of the circulatory system. KENNETH E. JOCHIM. *Dept. of Physiology, Univ. of Kansas, Lawrence.*

Pressure pulses were recorded with the Lilly capacitance manometer in the unopened carotid artery of the anesthetized dog simultaneously with flow pulses recorded with the electromagnetic flowmeter. Contour changes and alterations in the time relationships between the two pulses were studied after the administration of adrenaline and histamine. Multisection electrical networks were constructed as electrical analogs of the circulatory system; each section consisted of a shunt capacitance representing arterial elasticity and series resistance and inductance representing resistance to blood flow and effective blood mass respectively. Voltage and current pulses from various sections of the network were recorded oscillographically while the networks were energized with half-sine waves of current. Even when inductance was omitted from the networks, the current peaks preceded the voltage peaks, just as the flow peaks preceded the pressure peaks in the control records on dogs. Network parameters influencing this peak time relationship were studied. When inductance was included in the networks, the current peaks either preceded or followed the voltage peaks, depending on relative parameter values. In the animal, this relationship was changed by adrenaline and histamine, the latter drug causing the flow peak to be delayed so that in some cases it followed the pressure peak. Lowering the terminal resistance of the electrical network caused like changes. The significance of these similarities in behavior between the networks and the vascular system is discussed.

Further studies on cooling as a stimulus to smooth muscles. WILLIAM H. JOHNSON* AND JOHN F. PERKINS, JR. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

As shown previously (PERKINS *et al. Am. J. Physiol.* 163: 14, 1950) cooling alone acts as a stimulus to smooth muscles. The resulting 'cooling-response' curve, when obtained under isotonic

conditions with rapid cooling, is S-shaped and resembles a dose-response curve for a drug, except that it falls off with large amounts of cooling. In order to distinguish whether this was due to inability of the muscle to shorten, on the one hand, or whether to develop tension, isometric cooling-response curves were compared with isotonic. No significant difference was obtained in successful experiments on 5 retractor penis muscles (dog) and one pregnant uterus (rat). This suggests that extreme cooling is a less effective stimulus than moderate cooling, or else that the contractile system is less active. In another series of experiments, there was found to be a marked potentiating effect of potassium ions upon cooling alone as a stimulus. The threshold for cooling (i.e. smallest amount of cooling from 38°C. capable of evoking a response) was decreased in all experiments and the maximal height of contraction was increased, even with only slight increases in the concentration of potassium (from 2.7 m.Eq/l. = normal, to 5.4). With a K^+concentration of up to 10 times normal the muscle showed a marked decrease in resting length, but still responded to cooling. Upon rewarming, there was a marked though brief relaxation before regaining its high resting 'tone.'

Measurement of blood flow by conductivity method. R. J. JUSTER* AND R. C. INGRAHAM. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Work with the Stewart conductivity principle for blood flow measurement has resulted in technical changes and a further definition of the theoretical and practical problems involved. Design of electrical circuits was made so that the time-concentration curves of the injected salt were recorded as a single line by a direct writing ink oscillograph. The conductivity cell was made for placement outside of the artery thus avoiding the use of anticoagulants and difficulties arising when the electrode surface was in direct contact with the blood. It consists of a split-sleeve of lucite with silver plates fitting tightly over the artery, compressing it to just below diastolic size and permits the attainment of constant base lines. The recording of altered conductivity is not affected by variable distribution of the salt in the passing stream. In calibration, other investigators have had to deal arbitrarily with problems due to differences in the conductivity characteristics of blood when at rest and in motion. In this work a system was devised for calibrating on a moving stream of blood. Into the stream a rapid injection of saline was made and the time-concentration curve from a distant point was recorded while simultaneously the flow rate was directly measured. The mean height of the curve was measured and the salt concentration calculated for each determination. The regression coefficient of these two quantities established the calibration. Agreement between observed and calculated blood flows using this calibration method in a large series of tests averaged less than 10%.

Mass movement patterns in facilitation of neuromuscular responses. HERMAN KABAT AND MILTON GJELHAUG LEVINE* *Kabat-Kaiser Inst., Vallejo, Calif.*

Mass movement patterns have been applied to the therapy of paralysis. The use of such patterns in therapy facilitate the re-education of neuromuscular pathways. Evidence is presented for certain basic mechanisms which account for this facilitation.

Effects of anions on the mechanical responses of skeletal muscle. ARTHUR J. KAHN* AND ALEXANDER SANDOW. *Washington Square College of Arts and Sciences, New York Univ., New York City.*

In previous research (*Science* 112: 647, 1950) the ability of the nitrate ion very rapidly to produce potentiated maximal twitches of frog sartorii was reported. In the present studies (aided under contract with Office of Naval Research NR113-300) comparisons are made of maximal twitches obtained in chloride, bromide, nitrate and iodide Ringer's. Twitch duration and peak tension (T) of isometric twitches are increased by these anions in the order $Cl<Br<No_3<I$, with Br and I acting as rapidly as NO_3. The average percentage tension ratios are 100:155:235:310. Further, these anions all decrease the latent period time parameters to about the same extent. The tetanus tension output of nitrate-treated muscles is found to be 20–25% greater than that obtained for the same muscles in chloride Ringer's. Tension output of nitrate-treated muscles subjected to activity (40 maximal twitches/minute) is reduced to 25% of pre-activity tension in one-half the time required for a similar reduction in chloride Ringer's. Simulating nitrate tension in Cl-Ringer's muscles by spacing two stimuli $\frac{1}{50}$ second apart gives an activity series similar to that for the nitrate-treated muscle. The rapidity with which these ions potentiate twitch tension offers additional evidence for the conclusion, previously drawn only for NO_3, that they all influence contraction indirectly by way of a direct action on the excitatory membrane. The adherence of the relative effects of these anions to the lyotropic series suggests that their action is on the colloidal structure of the muscle cell membrane.

Hemolytic anemia produced by onions. M. KALSER* H. K. IVY* D. F. MAGEE* AND A. C. IVY. *Dept. of Clin. Science, Univ. of Illinois College of Medicine, Chicago.*

Dogs were fed both raw onions and various onion extracts. Raw onions fed in a dosage of 20 gm/kg/-day produced a 50% drop in hemoglobin and red cell count in an average of 15 days. The total white count showed a maximum elevation from 12,000 to 30,000 in 15 days. An oil extract was the most potent extract used, producing a 66% drop in hemoglobin and red cell count in 6–9 days. A marked leukocytosis was also found. Biliary fistula dogs fed the onion oil showed a similar lowering of red cells, hemoglobin, and hematocrit with a marked leukocytosis and reticulocytosis. The fragility increased from a starting hemolysis in 0.42% saline to 0.70% saline. Blood smears showed an increase in normoblasts, marked fragmentation, polychromatophilia, and anisocytosis. One dog after 5 daily doses of the extract developed a marked hemoglobinuria and died on the 6th day. His hemoglobin had dropped from 12–5 gm. and the red cell count from 5 million to 1.2 million. In these dogs the daily output of biliary bilirubin increased by 682%, the dog which died showing a tenfold increase in bilirubin. Much of the bilirubin was present as a suspension which settled out on standing.

Muscle and intravascular temperatures during exercise and their relation to pulmonary ventilation. FRED F. KAO* AND FRED S. GRODINS. *Dept. of Physiology, Northestern Univ. Med. School, Chicago, Ill.*

Pulmonary ventilation, oxygen consumption and temperatures in various locations were studied during exercise in barbitalized dogs. Exercise of one hindlimb was produced by percutaneous electrical stimulation using 60 cycle AC modulated sinusoidally at 100/minute. Temperatures were recorded by means of copper-constantan thermocouples from the following sites: the exercising muscle, subcutaneous tissue overlying the exercising muscle, carotid arterial blood and rectum. Control observations were made for 30 minutes, and the animals then exercised for 30 minutes. During the initial transient period, oxygen consumption and pulmonary ventilation rose very rapidly to reach peak values within 2 minutes, and then gradually diminished to reach steady state values approximately 10 minutes after the exercise had begun. During this same period, muscle temperature rose in a logarithmic fashion to reach a steady state at the same time as ventilation and oxygen consumption. Subcutaneous temperature followed muscle temperature with some lag, but did not reach a steady state within the period of observation. Both intracarotid and rectal temperatures showed an initial fall during the time when oxygen consumption, ventilation and muscle temperature were rising most rapidly. This was followed by a gradual rise which continued throughout the period of observation, i.e. a steady state was not reached. High and significant correlations were found between steady state values of muscle temperature and oxygen consumption ($r = 0.92$), and muscle temperature and pulmonary ventilation ($r = 0.87$).

Physical state of intracellular catalase. J. G. KAPLAN (introduced by C. B. WELD). *Dept. of Physiology, Dalhousie Univ., Halifax, Nova Scotia, Canada.*

At last year's meeting, the biological activity of crystalline liver catalase, following spreading as a monolayer at an air-water interface, was described. The surface-denatured enzyme differed from the original solution of native protein in four ways: *1*) its specific activity was lower, *2*) heat could increase its residual activity; *3*) it was highly resistant to heat inactivation and *4*) it was resistant to inactivation by ultra-violet irradiation. The conclusion was drawn that catalase activity was retained by the molecules which had actually undergone spreading and could not be attributed to molecules adsorbed to or enmeshed in the monolayer. In order to demonstrate the biological relevance of this work, erythrocyte catalase has been tested, both while still organized within the intact cell and after extraction from the lysed cell into solution. The activity of the intracellular enzyme was found to parallel in a striking manner that of the surface-unfolded enzyme in last year's *in vitro* experiments, whereas the extracted enzyme behaved like the solution of native crystalline liver catalase. The working hypothesis advanced to account for these facts is that the intra-cellular enzyme exists in an insoluble condition, unfolded at some cell interface; upon cytolysis, it is desorbed from the interface into solution in its native, rolled-up state. Evidence in favor of this hypothesis, both from experiments designed to put it to the test and from an extensive literature, has been accumulated. The significance of these findings and their bearing on cell structure and function are discussed.

Tobacco smoking and physical performance. PETER V. KARPOVICH AND CREIGHTON J. HALE.* *Dept. of Physiology, Springfield College, Springfield, Mass.*

Thirteen college students were used as subjects, 8 of them were habitual smokers, and 5 nonsmokers. Exercise consisted of performing on a bicycle ergometer a prescribed amount of work, comparable to a one-mile run on the track, in the shortest possible time (425 ped. revolutions with 8 lbs. of resistance or 48,928 foot-pounds of work). The experiment continued for 3½ to 5 months. Each subject served as his own control,

by either smoking or abstaining from smoking. The period of abstinence from smoking was from 7 to 10 days. The average riding time for all subjects but one was better when they did not smoke. This, however, was statistically significant for only 3 smokers and 2 nonsmokers, and was not significant for the group. Abstinence from smoking for one week showed improvement in a tobacco-sensitive subject, and no effect in nonsensitive ones. Reports made by the subjects regarding how they felt before and during the rides had no apparent connection with their performances. Since a relatively large proportion of an athletic team may consist of tobacco-sensitive men, the nonsmoking rule is a wise precaution.

Adrenal cortical requirements of adrenalectomized, x-irradiated rats. SEYMOUR KATSH AND ABRAHAM EDELMANN (introduced by LESLIE F. NIMS). *Biology Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Adrenalectomized male rats were maintained with whole gland adrenal extract (Upjohn), the amount being adjusted so that the animals had a slight weight gain during the period of observation. The animals were irradiated with 600 r of x-rays (160 kvp, 10 ma., 0.2 mm. Cu). It was found that the level of cortical extract had to be increased severalfold for 3–4 days after irradiation to inhibit weight loss. A gradual reduction in extract requirement then occurred, basal levels being reached after 5 days. This clearly indicates the need of the animal for increased adrenal hormones following x-irradiation. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Spontaneous hydrothorax in mice related to strain and diet. JOSEPH T. KING, Y. CHIUNG PUH LEE, CLAIRE C. CARR AND MAURICE B. VISSCHER. *Univ. of Minnesota, Minneapolis.*

Male mice of the $C_3H(Z)$ strain maintained for their lifetime on a partially purified diet adequate for rapid growth show an incidence of fatal hydrothorax of more than 50% when fed *ad libitum*. The maximum incidence is at the 14th month. Similar mice fed on fox chow do not show the disease, nor have females of this strain shown the phenomenon, but this problem is complicated by the high death rate from mammary cancer. Further, when male C_3H mice are fed the above partially purified diet modified so as to hold protein, minerals and vitamins constant, but reducing fat and carbohydrate to give a $\frac{1}{3}$-calorie restriction the hydrothorax does not occur. If previously calorie restricted male mice are changed over to *ad libitum* feeding they develop the disease. The hydrothorax is associated with hypoproteinemia, proteinuria, cardiac hypertrophy and frequently but not invariably with renal pathology. This syndrome has not been seen in an appreciable fraction of A strain mice, a large number of which have been studied under similar conditions.

Physiological aspects of electron-histology of heart. BRUNO KISCH. *Mt. Sinai Hospital, New York City.*

Electronmicroscopy performed with Miss Joan Bardet (Research Laboratories of Interchemical Corporation) shows that as a rule the myofibrils of the heart are isolated units, but their Z-bands are connected with each other. These fibrils are arranged in 'warp and woof' patterns. They are surrounded more abundantly by sarcoplasm than those of skeletal muscle. Nowhere do these myofibrils of the heart merge or show any syncytial arrangement; each is an isolated unit like the axon of a nerve. Whether or not all sarcoplasms merge seems also doubtful, because our pictures show the presence of a sarcolem, still doubted by some anatomists. Therefore the general syncytial concept of the heart muscle has to be changed to regard most or all of the myofibrils analogous to the neuraxons. However each contracting fibril must mechanically influence others connected with it by the woof of Z-bands. Where do the observed electrographic changes originate? Probably the initial sudden, quick change in the electrogram is connected with explosive changes in the contracting fibril. What happens later chemically there and within the sarcoplasm under influence of the granules and mitochondria and their enzymatic systems leads to restitution of the original relaxed condition. All this is represented by the slow ECG-phase from R until the end of T. Among the many new aspects to which electron-histology of the heart leads, the most impressive is that concerning the contractile units. There is no more justification to speak of a syncytial structure of these units than it would be to speak of a syncytium of the spinal nerves because they arise from the spinal cord out of two merging roots. The myofibril of the heart is an isolated functioning unit as is the nerve fiber.

Sleep pattern of infants. NATHANIEL KLEITMAN AND THEODORE G. ENGELMANN.* *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

From continuous actograms and protocols kept by their mothers on specially designed forms, the incidence and duration of sleep was followed in 19 infants, from the 3rd to the 26th week of life, under family home conditions. Even the earliest records revealed a diurnal disparity, the mean group duration of night sleep being 8.4 hours, as against 6.4 hours for day (usually 8 A.M.–8 P.M.).

Up to the 12th week, the total duration of sleep was *unchanged*, or slightly increased, but the night portion rose to 10.1 hours, at the expense of a corresponding fall in day sleep. For the succeeding 14 weeks, the duration of night sleep was *unchanged*, or slightly increased, but the day fraction continued to decrease, reaching 3.6 hours, for a total of 13.8 hours per 24 hours. Thus, not only were our objectively obtained figures much lower than those hitherto reported, but there was no marked diminution of sleep during the whole period of observation. The adjustment of the sleep-wakefulness pattern manifested itself mainly in a progressively more pronounced diurnal periodicity, culminating in one long unbroken period of night sleep and short morning and afternoon naps.

Defecation in animals following excision of Auerbach's plexus in the colon. FREDERICK M. KLINGE (introduced by H. L. WHITE). *Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

Nine adult cats were subjected to coeliotomy. From the distal colon 6–8 centimeters proximal to the pelvic peritoneal reflection, the longitudinal muscle layer, Auerbach's plexus, and the outer portion of the circular muscle layer were excised in intact sheets 1–6 centimeters in length. The dissections were carried around the entire circumference, transecting all blood and nerve supply entering via the mesentery. In several cats the proximal and distal circumferential incisions were carried to the tela submucosa in an effort to interrupt 'lateral' neural or muscular connections with the residual circular muscle. No attempt was made to transact possible connections between Meissner's plexus and the residual circular muscle. The residual circular muscle layer appeared intact and its blood supply adequate at the completion of the dissections. The denuded area was reperitonealized with omentum. Postoperatively, the cats have maintained excellent health on a diet of one-half pound of horse meat daily for 2–3 months. All cats have maintained stools of essentially normal size and frequency. All appear to be without abdominal distention. Histological section indicates complete removal of Auerbach's plexus. Two dogs, similarly subjected to 6-centimeter-long excisions of Auerbach's plexus in the distal colon, have maintained normal bowel movements and are without abdominal distention up to 6 weeks postoperatively.

The inner ear. Function of the Inner Ear (motion picture). H. G. KOBRAK AND J. E. HIND. *Otological Research Labs., Univ. of Chicago, Chicago, Ill.*

Some aspects of the function of the inner ear were studied cinematographically. Both fresh specimens and living animals were used. Using macroscopic-photographic techniques, low power magnification with good depth of field was achieved. *Statoreceptor:* The contents of the membranous labyrinth were made visible by artificially induced transparency of the wall of the membranous labyrinth (For a description of this method see KOBRAK, H. G. *Ann. Otol., Rhin., and Laryng.* 57: 1018, 1948.) Endolymphatic-fluid-movements were made visible by injection of fine silver particles. *Phonoreceptor:* Acoustic oscillations of various cochlear structures were photographed during auditory stimulation (stapes footplate, basilar membrane, intracochlear fluid). The acoustic reflex of the intratympanic muscles is demonstrated.

Effects of Dibenamine and cortin upon composition of blood plasma during acute radiation syndrome. HENRY I. KOHN. *Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md.*

On *day 0*, adult Sprague-Dawley rats received a single total-body exposure of 650 r (midbody-tissue dose, 250 kvp x-rays). One day before cardiac-blood sampling, food, but not water, was removed from the cages. The changes noted below were not due to radiation-induced alterations in food consumption. The plasma levels of sugar (normal, 140 mg.%) and NPN (normal, 32 mg.%) rose to 200 and 39 mg.%, respectively, during *days 0* to *4*. Total protein (normal, 6.7 gm.%) fell to 5.7 gm.% by *day 4*, returning to the pre-irradiation level by *day 12*. A factor disturbing the albumin-globulin ratio during *days 0* to *20* showed maximal activity on *day 4*. Phospholipid remained unchanged. Cholesterol (normal, 120 mg.%) rose to 190 mg.% by *day 4*, falling to normal or subnormal values after *day 7*. Chloride (normal, as NaCl, 605 mg.%) rose to 670 mg.% on *day 3*, remaining elevated until about *day 12*. The rise in cholesterol was blocked by subcutaneous Dibenamine or 2-dibenzylaminoethanol (70 mg/kg. 15 hours before, followed by 35 mg/kg. 33 hours after irradiation). The rise in both chloride and cholesterol was blocked by subcutaneous desoxycorticosterone acetate (2.5 mg/kg/day) or Upjohn lipoadrenal extract (1.25 ml/kg/day).

Protein constituents of canine pancreatic juice: electrophoretic and enzymatic studies. S. A. KOMAROV AND ALLAN L. GROSSBERG.* *Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.*

Pancreatic juice, secreted in response to a standard meal (300 gm. of lean beef heart), was obtained by canulation of the pancreatic duct in a dog with a chronic fistula, prepared according to Pavlov and Babkin. The juice, which contained from 150–300 mg. protein nitrogen per 100 ml., was dialyzed immediately after collection against distilled

water at 0°C. and lyophilized. Electrophoretic patterns of this protein material at a concentration of 1–3% in pH 8.6 diethylbarbiturate buffer, ionic strength 0.1, indicated the consistent presence of 6 components in relatively constant proportions. The results as to the number of components and their mobilities are in substantial accord with previous findings (MONRO AND THOMAS. *Am. J. Physiol.* 145/140, 1945–46; BYRNE, PHINNEY, SCHACTER AND YOUNG. *Abs. XIII Int. Physiol. Congress* 142, 1950). A partial separation of electrophoretic components was achieved and the resulting fractions were subjected to enzymatic analyses. No clean-cut separation of proteolytic activity was obtained, but a significant enrichment in the specific activity of some fractions was observed when Anson's method for trypsin was used with activation by enterokinase prepared according to Kunitz (*J. Gen. Physiol.* 22: 79, 1938).

Transacetylation in the choline acetylating system. SAUL R. KOREY (introduced by DAVID NACHMANSOHN). *Dept. of Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*

The enzymatic acetylation of choline in nervous tissue involves at least two steps: the transfer of an acyl group to Co. A or an enzyme acetylkinase and the subsequent esterification of choline by choline acetylase. The independence of these processes has been shown by inactivation of the acetylkinase component with the subsequent failure of the system to form acetylcholine and its reactivation by an acetyl activating enzyme. Transacetylation from O-acyl esters and α-acetyl amino acids has been shown to occur in the presence of the complete system. The origin of the acyl group participating in the esterification from the acetylated amino acids has been determined by the use of C^{14} labeled amino acids. The initial steps of the acetylation process have been investigated by means of thio and chlor substituted acetic acid. The results appear to indicate the significance of the electrophilic character of the carbonyl carbon in the reaction.

Segmental patterns in man. IRVIN M. KORR AND PRICE E. THOMAS.* *Dept. of Physiology, Kirksville College of Osteopathy and Surgery, Kirksville, Mo.*

All individuals studied have shown the presence of isolated segments or groups of segments characterized by lowered motor reflex thresholds of the spinal extensors (*Am. J. Physiol.* 105: 229, 1947) and areas of low electrical skin resistance (ESR) under conditions of rest (*Federation Proc.* 7: 67, 1948). The segmental patterns of motor reflex threshold and of ESR varied from individual to individual, but were consistent over periods of months, in a given individual. These findings were interpreted as signifying enduring segmental facilitation of the motor and sympathetic outflow in the involved segments. Relations to visceral, skeletal or myofascial disturbances and experimental variables were demonstrated (*Federation Proc.* 8: 87, 88, 1949). In the current investigations, in a large number of subjects, we have been mapping the topographical patterns of variation of the following features on the trunk: ESR, skin temperature, deep muscle temperature and depth-gradients, pain thresholds and areas of cutaneous hyperesthesia. In each modality we have found reproducible patterns which are characteristic of the subject. In a given subject, sharp deviations in the various entities (low ESR, altered temperature and gradients, low pain thresholds and hyperesthesia) show regional grouping. These findings, together with our earlier ones, indicate the presence in most individuals of segments or regions of the spinal cord which are distinguished from others by enduring imbalance of autonomic (vasomotor and sudomotor) activity, motor activity or pain sensitivity and combinations thereof. These segments are thought to be maintained in a state of imbalance by influences originating within the tissues of the corresponding segments.

Electromyographic and histological studies on animals made spastic by spinal cord ischemia. A. J. KOSMAN, JOHN HILL* AND RAY S. SNIDER. *Depts. of Physiology and Anatomy, Northwestern Univ. Med. School, Chicago, Ill.*

Electromyographic studies on cats and dogs made spastic by ischemia of the lumbosacral cord were carried out on the flexor and extensor muscle groups of the hind extremity. Histological examination of the cords were made in order to correlate degenerative cellular changes with the extent of the spasticity. For descriptive purposes the animals are divided into 3 groups: *group A*, rigidity; *group B*, strong spasticity; *group C*, mild spasticity. *Group-A* animals exhibited a pillar-like rigidity which persisted unabated for months. EMG records during this period showed a continuous and severe activity of both flexor and extensor muscle groups. Histological examination of the cord revealed massive cellular degeneration in the intermediate horn and central areas of the ventral horn. EMG records of *group-B* animals were characterized by occasional spontaneous discharges, exaggerated stretch reflexes with considerable after discharge, and hyperactive and repetitive tendon reactions. Motor deficits manifested themselves as partial paresis, over-stepping gait, and deficient proprioceptive placing. The principal

histological finding was a moderate gliosis of the central portion of the anterior horn involving chiefly the Type II Golgi cells. In *group C*, EMG records showed some increase in the stretch responses of the extensors only and hyperactive and repetitive tendon reactions. There were no manifest signs of motor impairment. Histologically, a mild gliosis of the central portion of the anterior horn was found.

Renal function following coarctation of the aorta in dogs. F. J. KOTTKE, W. G. KUBICEK AND R. L. VARCO.* *Div. of Physical Medicine and Dept. of Surgery, Univ. of Minnesota Med. School, Minneapolis.*

Following artificial coarctation of the aorta of dogs, hypertension may develop distal as well as proximal to the aortic constriction. The change in renal hemodynamics following coarctation would appear to be similar to that occurring following the application of Goldblatt clamps to the renal arteries. In such case removal of the coarctation might be expected to cause the blood pressure to revert to a normal level. Therefore studies have been undertaken to elucidate the effect of coarctation of the aorta on renal hemodynamics and function. Marked constriction of the aorta was produced in young puppies by partial ligation. Growth and fibrosis caused further increases in relative and absolute constriction. Hypertension developed caudad as well as cephalad to the constriction in varying degree in the series of dogs. The carotid pulse pressure was augmented markedly while the femoral pulse pressure was severely damped. High mean femoral blood pressures were obtained although the femoral pulse was reduced. Renal plasma flow, glomerular filtration, tubular excretion of paraminohippurate, and tubular reabsorption of glucose were studied in repeated tests on 9 dogs with coarctation. These values as well as the plasma filtration fraction and C_{PAH}/T_{mPAH} ratio were within the range of normal for dogs.

Role of carbon dioxide in acute oxygen toxicity at 3½ atmospheres inspired oxygen tension. R. H. KOUGH, C. J. LAMBERTSEN, M. W. STROUD, 3rd, R. A. GOULD AND J. H. EWING (introduced by A. N. RICHARDS). *Depts. of Pharmacology, Medicine and Psychiatry, Univ. of Pennsylvania, Philadelphia.*

Four types of experiments were performed in man at 3½ atm. inspired oxygen (OHP). In 4 men the highest pCO_2 found in subcutaneous gas depots was 50 mm. Hg. In 12, internal jugular pCO_2 increased 3 mm. Hg while arterial pCO_2 decreased 5 mm. In 7, cerebral blood flow (CBF) by the N_2O method decreased 25%; cerebral vascular resistance increased 50%; and cerebral oxygen consumption was unchanged so that the internal jugular pO_2 was only 75 mm. Hg. In man, therefore, it appears that any elevation of tissue pCO_2 accompanying acute oxygen poisoning at OHP is adequately explained by loss of the dual function of hemoglobin and changes in blood flow and is not sufficient *per se* to produce convulsions. The significant finding in 7 experiments on 4 who breathed 2% CO_2 in O_2 at 3½ atm. was a decrease in the A-V O_2 difference resulting in an increase in the internal jugular pO_2 to a mean value of 800 mm. Hg. The breathing of CO_2 may conceivably hasten the onset of oxygen convulsions by increasing the CBF so that a larger bulk of the brain is exposed to higher, and presumably more toxic, levels of oxygen tension.

Urinary calcium in guinea pigs on diets deficient in the anti-stiffness factor. HUGO KRUEGER, ROSALIND WULZEN, LLOYD YONCE* DARRELL DAVIS* AND PHILLIP LEVEQUE.* *Oregon State College, Corvallis.*

Guinea pigs on diets deficient in the anti-stiffness factor display many unusual calcium pathologies. DASF guinea pigs also yield a much greater volume of urine than do guinea pigs on the standard Oregon State College stock diets. Guinea pigs taken from the standard stock diet and placed in metabolism cages with no food but water *ad libitum* excrete 4 to 22 mg. (average of 30 experiments 10.5 mg.) of calcium in a 36-hour period. Calcium excretion is usually higher in the earlier periods. In 23% of the animals on the standard diet over one-half of the calcium was excreted in the first 6-hour periods. About one-fourth of the calcium excretion curves resembled truncated rectangular hyperbolae. DASF guinea pigs excreted much more calcium with a range of 9 to 73 mg. during the 36 hours and an average of 35.2 mg. in 30 experiments. Calcium excretion was exceedingly high in the first 6-hour period and over 50% of the calcium excreted during the 36 hours was obtained during the first 6 hours in 72% of the deficient guinea pigs. Ninety per cent of the calcium excretion curves resembled truncated rectangular hyperbolae and the remaining 10% could be considered as segments of rectangular hyperbolae with a much less acute curvature. Are the differences in calcium excretion due to the skim milk diet per se or to the deficiency in the anti-stiffness factor?

Stress-induced lymphocytosis in adrenalectomized mice. L. F. KUMAGAI* AND T. F. DOUGHERTY. *Dept. of Anatomy, Univ. of Utah, Salt Lake City.*

Time relationship and extent of alterations in lymphatic organ size and circulating lymphocytes were compared in stressed and non-stressed adrenalectomized CBA mice. Stress stimuli were starva-

tion, histamine and anaphylaxis. Lymphocytosis appeared 24 hours after adrenalectomy in non-stressed mice and maintained a constant level for 5 days of study. Animals stressed 2 hours after adrenalectomy had lymphocytosis 2 hours later, rising to enormous levels at 8 hours and returning to the level found in adrenalectomized non-stressed animals at 24 hours. This lymphocytotic response in adrenalectomized mice is the inverse image of the 'lymphopenic response' of intact animals. After adrenalectomy, lymphatic organs of mice in sublethal anaphylaxis were significantly larger than those of non-stressed animals at comparable time intervals. The lymphocytosis of stress occurred also in splenectomized mice and, thus, was not due to splenic contraction alone. Enhanced proliferation could partially account for the lymphocytosis, since mitotic indices were greater in stressed than in non-stressed animals. Stress may more rapidly deplete adrenocortical hormone remaining after adrenalectomy, thus shifting the balance from lymphocytolysis to proliferation. This explanation is supported by experiments indicating that the degree of lymphocytosis in adrenalectomized mice maintained on varied dosages of cortisone acetate is a function of two factors—the strength of the stress stimulus and the amount of hormone available. Thus a lymphocytosis may indicate that the amount of hormone is insufficient to maintain simultaneously normal numbers of lymphocytes and to meet the hormone requirement raised by stress. (Supported by Committee on Growth of the National Research Council.)

Nucleic acid and phosphate changes following sinus gland removal. ELOISE KUNTZ (introduced by RUTH CONKLIN). *Marine Biological Lab. and Vassar College, Poughkeepsie, N. Y.*

Crustacean sinus glands are endocrine organs which influence growth and metabolic pattern. In the present investigation the composition of the hepatopancreas of blue crabs (*Callinectes sapidus*) from which the sinus glands had been removed was compared with that of normal crabs. The type of change was influenced by the environmental conditions. A marked increase in nucleic acids and acid soluble phosphates was observed in those operated animals which were kept in outdoor live cars. Sinus glandless crabs kept in indoor aquaria showed a decrease in nucleic acid concentration. This is discussed in relation to growth and the ability to respond to environmental stresses.

A technique for perfusion of isolated kidney. SHERMAN KUPFER (introduced by ROBERT M. BIRD). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

Studies of heart-lung-kidney preparations have been limited by the development of heart failure and pulmonary edema. Substitution of a pump and valves for the heart led to hemolysis. Glomerular filtration rates have been reported only in the latter preparation and have been found to be uniformly low. It was felt that a pump-lung-kidney preparation might be more easily prepared and controlled than a heart-lung-kidney preparation, providing a non-hemolyzing pump could be devised. A pump consisting of a series of fingers which incompletely compress a short length of rubber tubing in a sine-wave motion has proven satisfactory. The output as well as the pulse pressure can be altered by varying the degree of compression. Pulse pressure can also be varied by means of an air chamber. Blood flow from the renal vein is measured continuously by a flowmeter and intermittently in a graduate. Blood is heparinized; arterialization is accomplished by intermittent inflation of the lungs with a 95% O_2–5% CO_2 mixture. The determinations of creatinine and PAH clearances and the rate of electrolyte excretion are performed in the standard manner. The effects of various drugs and hormones upon the isolated kidney are now being studied. (Aided by a grant from the U. S. Public Health Service.)

Polymerization of actin. K. LAKI (introduced by L. MARSHALL). *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

It has been found that actin preparations contain ATP. Part of this ATP disappears during polymerization of actin (STRAUB AND FEUER, *Biochimica et Biophysica Acta* 4: 455, 1950; LAKI, BOWEN AND CLARK, *J. Gen. Physiol.* 33: 437, 1950). Employing the method of Kalckar (*J. Biol. Chem.* 167: 461, 1947) to estimate ATP, ADP, AMP, and the method of Kornberg (*J. Biol. Chem.* 182: 805, 1950) to estimate ATP and ADP, it was found that, besides ATP, actin contains ADP and AMP as well. It was also found that during polymerization of actin there is an increase in the ADP concentration amounting up to 82% of the ATP disappearance. Corresponding to these findings and in contrast to Dubuisson and Mathieu's report (*Experientia* 6: 103, 1950), an increase in inorganic phosphate and a decrease in labile phosphate content occurs during polymerization. Since no ATPase has been found in these actin preparations, these findings support Straub and Feuer's view that during polymerization, ATP is dephosphorylated to ADP and that this process is connected with the polymerization process. Experiments in progress, however, do not support Straub and Feuer's theory that during the depolymerization of actin, ATP is restored from ADP and in-

organic phosphate. While actin preparations contain a fairly active adenylic acid deaminase, no ATP deaminase is found in contrast to the assumption of Snellman and Gelotte (*Exper. Cell Research* 1: 234, 1950).

Effect of heat and ultrasound on conduction in bullfrog nerve. E. H. LAMBERT, W. J. TREANOR* AND J. F. HERRICK. *Mayo Fndn., Rochester, Minn.*

The effects of heat and ultrasound on the various fiber components of the sciatic nerve of *Rana catesbiana* have been compared. Heat was applied by a thermode, 1 cm. long, at the center of the nerve in a paraffin oil bath. With a slow rate of heating, block of A fibers began on the average at 41°C. and became complete or nearly complete at 43° to 44° in the order, γ, β, α. With continued heating recovery of conduction occurred in 30 to 100% of fibers at 44° to 45°. Block again became complete at 45° to 46° in the order, α, β, γ. With a more rapid rate of heating the initial block and recovery were often not observed. B and C fibers were completely blocked at 43.7° and 45.2°, respectively. Neither showed recovery with continued heating. Susceptibility to irreversible block by heating was in the order, A, C, B. By successive heating, nerves with only B fibers conducting could be prepared. Ultrasound had no effect on nerve conduction unless the temperature of the nerve was allowed to increase. Under these conditions block occurred at approximately the same temperature as it did in the thermode. Certain differences in susceptibility of fiber groups to block by heating with thermode and ultrasound may be due to differences in rate and area of heating.

A new method for the study of vessel characteristics. Pressure-distensibility relationship of human arteries. MILTON LANDOWNE. *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and Section on Gerontology, Baltimore City Hospitals, Baltimore, Md.*

Conventional pulse wave studies derive velocity of progagation at diastolic pressures only, and usually by recording the movement of tissues adjacent to the vessel. In preliminary experiments with a new method, intra-arterial radial pressures are recorded photographically using a sensitive capacitance type manometer system adequate for frequencies up to 50 cycles/second. Single brief impacts at a rate of approximately 10/second are delivered to the skin over the artery at midhumerus. A signal records the moment of impact. Primary impact waves with a frequency of over 20 cycles/second and an amplitude of 10–30 mm. Hg may be recorded from the radial artery superimposed upon the pressure pulse. Several hundred precise observations may be obtained in less than one minute. Pressures below diastolic are achieved by short periods of proximal arterial occlusion. An instrumental 'zero' is obtained by placing the impactor immediately proximal to the radial needle. Net transmission times (t) are measured to the nearest msec. and grouped according to pressure (p). The absolute reliability of velocity ($1/t$) is reduced at higher pressures. The method has been critically tested for validity and reproducibility. The explanations which have been variously proposed for wave progagation in large arteries do not appear to fit either the individual or mean curves obtained. As an illustration, in 20 males in the seventh decade, mean transmission time may be approximated as $\bar{t}$ (sec/m.) = 0.41 $\bar{p}^{-0.43}$ (mm.Hg).

Observations on adrenal glands of dogs following hypophysectomy. N. LANE (introduced by L. W. EICHNA). *Dept. of Pathology, Univ. Hosp., and Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

It has been shown by de Bodo *et al.* (*Federation Proc.* 9: 30, 1950) and Schwartz *et al.* (*Federation Proc.* this issue) that the hypophysectomized dog responds to sodium restriction and administration of potassium load in a manner comparable to a normal animal, but shows an impaired ability to excrete water load. Associated morphological observations were directed toward establishing the degree of completeness of hypophysectomy and evaluating the atrophic changes in the adrenal cortex. By serial section technique, the 5 dogs to be reported were shown to be totally lacking in adenohypophyseal tissue (pars distalis, pars tuberalis, pars intermedia). Study of the adrenal glands uniformly showed marked reduction of weight and changes of an atrophic nature in the cortex, including the zona glomerulosa. These consisted of reduced cell size and decreased cytoplasmic vacuolization and were qualitatively comparable to those seen in the zona fasciculata. Confirmatory quantitative data regarding cell measurements are being compiled. These observations in conjunction with the finding of impaired ability to excrete water load militate against the existence of an autonomous zona glomerulosa. They indicate, rather, that all 3 cortical layers are under trophic pituitary control. In addition, the observations do not suggest that particular functions of the adrenal gland are limited to certain zones.

Disynaptic reflex linkage between muscles of a myotatic unit. YVES LAPORTE* AND DAVID P. C. LLOYD. *Rockefeller Inst., New York City.*

Monosynaptic conditioning of motoneurons by low-threshold (*group I*) afferent fibers of muscle origin is gauged by the finding of alteration in monosynaptic test reflexes beginning when condi-

tioning and test volleys converge in synchrony. By monosynaptic action *group I* afferent fibers from a given muscle facilitate response of its synergists and inhibit response of its antagonists. Facilitatory and inhibitory actions decay exponentially to 1/e in 4 msec. Within the same low-threshold group are afferent fibers that, after a central latency of 0.5–0.6 msec., inhibit the action of synergists and facilitate the action of antagonists. Internuncial relay accounts for the delay. Although monosynaptic conditioning can be isolated from the relayed, presumably by failure of the latter to negotiate the internuncial synapse, segregation in reverse has not been achieved. Thus relayed conditioning is written upon the monosynaptic described above, its onset signaled by sharp divergence at 0.5–0.6 msec. from actual and anticipated course of the latter. Participation in the reactions discussed of the higher threshold *group II* afferent fibers is contraindicated on three grounds: threshold, which is too low; latency, which is too short; and by the fact that *group II* fibers in action facilitate flexors and inhibit extensors whether they be synergists, antagonists or independents. Monosynaptic pathways have been shown to serve the myotatic reflex. The relayed *group I* pathways, being in pattern of distribution precisely inverse to the monosynaptic, are well suited to the role of countermanding operation of the myotatic reflex. Hence they are proposed as the pathways for mediation of the lengthening reaction and its associated excitation of antagonists.

Glucose requirements of sympathetic ganglia. MARTIN G. LARRABEE, CHARLES EDWARDS* AND JUAN GARCIA RAMOS.* *Biophysics Lab., Johns Hopkins Univ. and Hosp., Baltimore, Md.*

Excised superior cervical ganglia of rats and rabbits were bathed in a solution which contained sodium, potassium, calcium, magnesium, chloride, phosphate, and glucose (1 gm/l.) and was equilibrated with 5% 00_2, 95% O_2. Transmission of nerve impulses was tested electrically. Rate of oxygen consumption was determined by measuring the oxygen concentration within a fluid-filled respirometer by means of a polarized platinum electrode. When the bathing solution was changed to one containing no glucose, transmission did not fail and rate of oxygen consumption was unchanged for one or more hours, provided the preganglionic nerve was stimulated only infrequently. During stimulation at a frequency of 12/second in the absence of glucose, the postganglionic action potential declined to less than 25% of its initial height in less than 20 minutes, and recovered after addition of glucose even with continued stimulation. The relative sensibility of nerve fibers and synaptic structures to glucose-lack was investigated at various frequencies of activity. For further investigations of glucose requirements methods were developed for measuring glucose uptake. An excised rabbit ganglion was bathed in 0.02 ml. of solution, which was raised and lowered by a piston to expose the ganglion alternately to oxygen and to solution. Solution was subsequently withdrawn and analyzed for residual glucose. This was done by drying the sample, redissolving in 0.05 ml. of oxygenated solution containing glucose oxidase, and finally measuring the concentration of oxygen remaining after the glucose had become oxidized.

Measurements on elasticity of isolated rat lung. RICHARD W. LAWTON AND DOYLE JOSLIN (introduced by DAYTON J. EDWARDS). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

The elasticity of the isolated exsanguinated rat lung preparation has been studied by means of volume-pressure curves obtained by both positive intratracheal pressure inflation and by subatmospheric extrapleural pressure inflation. Inflation was carried out in an incremental manner and also continuously at inflation rates of 1–40 mm. water pressure per minute. In addition, the retractile behavior of the lung has been studied following both types of inflation. The volume-pressure curves and the retractile characteristics observed under these circumstances indicate that different elastic responses to distention may be ascribed to the bronchial system and the air spaces, the former being relatively stiff and highly retractile and the latter showing high distensibility, plastic flow and rapid creep. The possibility of gaseous diffusion is enhanced in air space inflation. At the more rapid rates of subatmospheric extrapleural pressure inflation the volume-pressure curve becomes less dependent on the inflation rate, the contributions of the separate anatomical structures of the lung to the pressure-volume curve tend to become fused and reversibility is enhanced.

Influence of hypophysectomy, thyroidectomy and low dietary protein on plasma and liver proteins in thiouracil-fed rats. JAMES H. LEATHEM. *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.*

An increase in liver protein and in plasma globulin concentration follows the feeding of 0.5% thiouracil to normal or adrenalectomized adult male rats for 20 days. In the current study, adult male rats were hypophysectomized and fed 0.5% thiouracil immediately for comparison with operated and normal pair fed controls. Thiouracil prevented liver weight and protein loss after hypophysectomy although all other organs exhibited typical atrophy. The increase in plasma

globulin concentration following hypophysectomy was not enhanced by thiouracil but plasma nonprotein nitrogen was increased. Thiouracil feeding to thyroidectomized rats retained liver weight and relative liver protein content slightly above that of pair fed thyroidectomized controls. Thiouracil significantly increased plasma globulin concentration in thyroidectomized rats. The influence of a low dietary protein level on the actions of thiouracil was studied in rats fed a 6% casein diet for 20 days. Thiouracil increased liver weight and relative liver protein to above normal levels despite the low protein diet. The effect was specific as kidney and testis protein content was not increased by thiouracil. The 6% protein diet did not prevent the thyroid hypertrophy anticipated with thiouracil.

Relationship of skeletal muscle tone to carotid blood pressure. CHESTER E. LEESE AND JAMES T. HICKS.* *Dept. of Physiology, School of Medicine, George Washington Univ., Washington, D. C.*

The literature suggests that muscle tone and carotid blood pressure vary directly. To test the hypothesis 153 tests were made on 25 cats. The carotid blood pressures and the isometric contractions of the soleus and gastrocnemius muscles were measured for the first two minutes following stimulation of the peripheral stump of the cut sciatic nerve. The area of the blood pressure and muscle tensions were measured by a planimeter which gave values in square centimeters. Correlation was positive, and 'r' was equal to .281. Several experimental methods for raising and lowering the carotid pressure were tried. A positive correlation was found when the pressor action of small doses of pitressin and epinephrine were studied. Large doses of epinephrine resulted in a negative correlation. The depressor actions of mechalyl and histamine were studied, and it was found that as carotid pressure area decreased, so did the muscle tension area. The correlations were positive.

Competition among monoses for active transport into the human red cell. PAUL G. LEFEVRE AND ROBERT I. DAVIES.* *Dept. of Physiology and Biophysics, Univ. of Vermont, Burlington.*

The kinetics of osmotic volume changes and the influence thereon of chemical inhibitors previously suggested an active transport of glucose (*d*-dextrose) into the human erythrocyte. To ascertain the extent of applicability of this system, similar experiments were undertaken with the following related substances: hexoses *d*-levulose, *d*-galactose, *l*-sorbose, and *d*-mannose, pentoses *d*-xylose and *l*-arabinose, and the alcohols adonitol, *d*-mannitol, and *i*-inositol. Each aldose showed the pattern previously described for glucose, in the relation between its concentration and its rate of penetration, suggesting some rate-limiting factor in the penetrating mechanism. The two ketoses behaved approximately according to Fick's law (suggesting passive diffusion), while the alcohols did not perceptibly penetrate. Yet the entrance of both aldoses and ketoses was inhibited by Hg^{++} or by *p*-chloromercuribenzoate, at *ca.* 10^{-5} M, and all showed a high Q_{10}; ketose uptake was specifically sensitive to inhibition by barbital. In mixtures of two sugars at equal concentrations, any aldose stopped or greatly retarded entry of either ketose, while the ketose did not perceptibly affect aldose uptake. Within each class, similar contrasts in mutual inhibitory potency were evident. These relative potencies were also reflected in the dilutions at which each sugar effected a given degree of inhibition of the entry of a less potent member of the series. All results can be interpreted in terms of a carrier system shared by all the tested sugars, on the basis of differences in the dissociation constant of the complexes formed by the several sugars with the supposed carrier.

Combined action of metrazol and aranthol in pentothal poisoning. ALFRED LEIMDORFER. *Dept. of Pharmacology, Stritch School of Medicine of Loyola Univ., Chicago, Ill.*

Pentothal sodium was slowly injected i.v. into 15 dogs until the respiration stopped. Under these conditions several stages due to anoxia could be distinguished: *1*) a rise in blood pressure; *2*) cardiac disturbances (extrasystoles and S-T depression, seen in the electrocardiogram, and lowering of the blood pressure); *3*) finally a cardiovascular collapse with severe fall in blood pressure. In attempts to treat this poisoning it was very important to combat the respiratory depression and simultaneously the cardiovascular disturbances. Thus Metrazol (30–40 mg/kg.) was injected i.v. These amounts of Metrazol were sufficient to start the respiration (without producing convulsions and a subsequent depression of the respiration. However, after Metrazol the ECG disturbances usually did not disappear and the blood pressure fell slightly for a short time. On the other hand, after Aranthol (an aliphatic amine injected in the amounts of 5–10 mg/kg. a few minutes after Metrazol) the extrasystoles disappeared instantaneously and the other ECG disturbances changed slowly toward normal. The blood pressure was greatly increased after Aranthol for 5–10 minutes and returned then slowly to the level before Pentothal injection. There was a remarkable improvement of the respiration in depth and rate (due to the improved circulation of the respiratory center). Usually about 30–40 minutes after the Pentothal poisoning the dogs awakened and

responded to pain stimuli. In states of very severe cardiovascular collapse when the heart had already stopped beating it was essential to inject adrenaline in order to initiate cardiovascular activity. Aranthol was then administered for its longer lasting cardiovascular action.

Neurospora mutants requiring acetate for growth. JOSEPH LEIN, DOUGLAS C. APPLEBY AND PATRICIA S. LEIN, (introduced by ROBERT GAUNT). *Dept. of Zoology, Syracuse Univ., Syracuse, New York.*

Four Neurospora mutants which required acetate for growth were isolated. Heterocaryotic tests showed that each of these mutations involved different genes. While no growth occurred in media in which sucrose or glucose was the carbon source, a small amount of growth occurred if glycerine, arabinose, lactose and glycine were used. When small amounts of glucose were added in addition to these carbon sources, growth was prevented. This evidence is thought to indicate that in these mutants some product of glucose metabolism is inhibiting a reaction involved in acetate synthesis and this inhibition is the cause of the acetate requirement. Three of the mutants grow well when minimal medium, containing sucrose as a carbon source, is supplemented with acetate. The fourth mutant grows poorly under these conditions. It was found that the poor growth of the latter mutant is due to inhibition of acetate utilization by sucrose or some metabolic product derived from it. If this mutant is grown on xylose as the carbon source with an acetate supplement, luxuriant growth occurs. Using chromatographic analysis, it was shown that three of the mutants grown in minimal medium supplemented with acetate accumulate pyruvic acid in large quantities. Various substances were tested to determine if they could substitute for acetate with these mutants. Pyruvic acid, in large concentrations, did promote some growth although it was not as effective as acetate. No other substance tried was effective.

Role of temperature gradients within ventricular muscle in genesis of normal T wave of electrocardiogram and ventricular gradient responsible for it. EUGENE LEPESCHKIN (introduced by A. H. CHAMBERS). *Div. of Exptl. Medicine, Univ. of Vermont, Burlington.*

The temperature of the ventricular surface, cavities and muscle layers of 10 dogs was measured by means of very thin, pliable copper-constantan thermocouples. The temperatures were correlated with unipolar direct electrograms from the regions where the temperature was measured. After restoration of the chest wall, the temperature of the intraventricular blood was found to be up to 1°C. lower and that of the epicardium 0.5–1.0°C. lower than the intramyocardial temperature. There was a highly significant correlation between this negative temperature gradient and the negative values of the ventricular gradient (area of the QRS-T complex, determined planimetrically) in intracavitary leads from the right and left ventricles. The correlation was less significant between the epicardial temperature and the local ventricular gradient in different portions of the heart surface; in fact, the diaphragmatic ventricular surface showed highest temperatures but sometimes had a negative ventricular gradient. When Ringer's solution of exactly the same temperature as the right intracavitary blood was applied to the surface of the right ventricle, the ventricular gradient in the unipolar lead from the point of application became zero. The tentative conclusion from these experiments, which are being continued, is that the normal T wave and the normal electrical ventricular gradient depend on the temperature difference between the trabecular portion of the subendocardial ventricular layers in the apical region, which are surrounded by blood, and the subepicardial solid portions of the myocardium in these regions.

Electrolyte losses in edematous individuals following organic mercurials. GERSON T. LESSER, MARCELLE F. DUNNING, FREDERICK H. EPSTEIN AND EUGENE Y. BERGER (introduced by J. MURRAY STEELE). *New York Univ. Research Service,* Goldwater Memorial Hosp. and Dept. of Medicine, New York Univ. *Med. School, New York City.*

Seventy-five mercurial diureses were studied in 17 patients with congestive heart failure or cirrhosis of the liver who were accumulating fluid. In order to estimate losses attributable to the mercurial alone the water and electrolyte excreted the day prior to mercurial administration was subtracted from that excreted the day of the mercurial. The average loss of water was 2.9 liters. The average loss of chloride was 426, of sodium 281, and of potassium 92 mEq. Potassium depletion may account for such clinical symptoms as the weakness or 'digitalis toxicity' seen after mercurial diuresis. The fluid lost attributable to the mercurial diuresis is isotonic with body fluids. Mean concentrations of electrolyte in the fluid lost were: chloride 151, sodium 97, and potassium 35 mEq/l. Changes in the concentration of plasma chloride 24 hours after mercurial administration (−1 to −10 mEq/l.) would indicate that chloride and water loss originate from extracellular fluid. Change in plasma potassium (0 to −1.5 mEq/l.) was by no means enough to account for the potassium excreted so that intracellular loss of this cation is evident. Plasma sodium concentration

into the cell in exchange for potassium. Thus did not change, indicating that sodium had shifted mercurial diuresis in a patient who is accumulating fluid leaves him relatively alkalotic, with a loss of intracellular potassium and a gain in intracellular sodium.

Variation in phosphorus compounds during adult development of the blowfly, Calliphora erythrocephala. L. LEVENBOOK (introduced by CARROLL M. WILLIAMS). *Biological Labs., Harvard Univ., Cambridge, Mass.*

An investigation has been made of phosphorus-containing compounds of the blowfly during its metamorphosis. Inorganic-P and P-esters extracted by trichloroacetic acid from pupae of known ages were examined by acid hydrolysis before and after precipitation with barium and ethanol. The residue was used for analysis of lipid-P, nucleoprotein-P, and protein-P. No metaphosphate, inorganic pyrophosphate, or protein-P was found. Total acid-soluble-P remained constant throughout development (75–78 mg.P/100 gm. wet wt.). True inorganic P was minimal at the onset of puparium formation (13), but increased rapidly during the following few hours to 30, and then more gradually to a maximum (47) half way through development. Thereafter it showed a progressive decrease. ATP-like compounds (hydrolyzing in 10 minutes), and difficultly hydrolyzable-P (e.g. glucose-6-P, phosphoglyceric acid), were maximal at the commencement of puparium formation (40 and 20, respectively). Difficulty hydrolyzable-P decreased progressively throughout metamorphosis, whereas ATP-like compounds followed a **U**-shaped curve varying inversely with the inorganic P. Other acid-soluble-P compounds were at all times low (0–7). An unidentified, labile, apparently new P-compound, hydrolyzing completely in 2.5 N acid in 15–20 minutes at 20°C., was present in highest titer (15.5) at the onset of puparium formation, but disappeared almost completely by the time of adult emergence. Phospholipid-P and nucleoprotein-P remained approximately constant throughout development (36–46, and 60–72, respectively). At all stages pentose nucleic acid accounted for > 90% of the total nucleic acid. (Aided by American Cancer Society.)

Genesis of electrocardiographic pattern of digitalis. H. LEVINE, H. M. GELLER, R. S. SIKAND AND L. H. NAHUM (introduced by J. S. NICHOLAS). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The suggestion has been made that the digitalis pattern is associated with QT shortening, indicating an acceleration of the repolarization of the slowest fibers. Validity of QT measurements must therefore be considered. Multiple simultaneous tracings have demonstrated that the earliest Q and latest T are found only in the left precordial lead, iso-electric intervals being present in the other leads. Thus the 'absolute QT' must be measured in the left precordial lead. Cedilanid was injected intravenously in large doses in dogs, and serial electrocardiograms taken over 24-hour periods. When absolute QT was measured, no shortening appeared. The most uniform and the earliest evidence of digitalis activity was inversion of T in the left precordial lead, indicating that the apical fibers were most markedly affected by digitalis. The absence of QT shortening suggests that when the T wave is inverted, the repolarization of the region beneath the electrode is slowed. Since repolarization of the apical fibers was delayed, procedures to accelerate repolarization after digitalization, such as application of physical heat to the entire body and intravenous injection of KCl, were carried out. Whenever repolarization was hastened, the digitalis pattern disappeared. On the other hand, when repolarization was slowed by such procedures as cooling, the pattern remained unchanged. It may be concluded that the digitalis pattern as it develops in the normal dog is due to delayed repolarization of the apical fibers.

Influence of cortisone and ACTH on water and electrolyte distribution and renal function. MARVIN F. LEVITT AND MORTIMER E. Bader (introduced by HOMER W. SMITH). *Depts. of Medicine and Pediatrics, Mt. Sinai Hospital, New York City.*

The influence of cortisone and ACTH on body water and electrolyte distribution was studied in patients maintained in approximate water, sodium, and chloride balance (salt-free diet). Five patients were fed a rice diet (containing 5–7 mEq. of sodium, 10 mEq. of chloride, and 40 mEq. of potassium/day) for a 3–7 day control period and during 9–23 days of treatment with 100–200 mg. of cortisone or 100 mg. of ACTH daily. Serial measurements of body weight, plasma volume, inulin space of distribution (extracellular volume), serum electrolyte concentration, urinary electrolyte excretion, glomerular filtration rate and renal plasma flow were made before and during therapy. Despite the rigid sodium and chloride restriction, cortisone and ACTH induced in each patient a progressive increase of 25–40% in isotonic extracellular volume. This volume reached its peak after 8–9 days of hormone treatment and then decreased to normal despite the continuation of therapy. The changes in total extracellular water, sodium and chloride were not reflected in body weight or exogenous sodium and chloride balances. Coincident with the maximal increase in extracellular volume, plasma volume increased 5–12%. Renal function evidenced first a progressive rise and subsequently a fall in glomerular filtration rate and

filtration fraction. The peak changes in extracellular volume and renal function usually coincided. These data imply that cortisone and ACTH produce a temporary shift of sodium, chloride and water into the measurable extracellular volume.

Effect of hypertonic saline upon the renal tubular transport mechanisms for sodium glucose and PAH in dogs. MATTHEW N. LEVY, JAY ANKENEY AND PIERRE JEANNERET (introduced by DAVID F. OPDYKE). *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio.*

After a pair of control clearance periods, 3% saline was administered intravenously to pentobarbitalized dogs at gradually increasing rates. Creatinine and sodium clearances were determined throughout all experiments, while glucose Tm was computed in half the experiments; PAH Tm in the remainder. As the plasma sodium concentration rose, the glomerular filtration rate remained fairly constant. Owing to the elevated plasma sodium level, the rate of sodium excretion increased markedly, but sodium reabsorption was maintained at a constant rate. Tubular function was impaired by the hypertonic saline to the extent that Tm's for glucose and PAH were depressed below control values, PAH transport to a greater extent than glucose. PAH Tm's decreased from 43 to 58% of control as the result of hypertonic saline administration, while glucose Tm's varied from 64 to 105% of control at equivalent plasma sodium concentrations.

Mitral valve orifice areas and blood flows as calculated from hydraulic formulae. BENJAMIN M. LEWIS,* FLORENCE W. HAYNES AND RALPH J. SPEIGL.* *Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

By means of hydraulic principles, formulae have been derived for estimating the area of the mitral regurgitant orifice (MRA) and the amount of blood regurgitating through it (Fr.). Because of the numerous indirect measurements in man, values presented serve as an index of regurgitant orifice areas and flows. In 3 patients studied by the technique of cardiac catheterization, mitral valve stenotic areas were measured at operation to be 1.6, 1.7, and 1.3 $cm.^2$; MRA's were 0.3, 0.3, and 0.4 $cm.^2$ respectively, as calculated from the formulae. These values were checked at rest, on exercise, and on recovery from exercise in all 3 studies. Resting regurgitant blood flows ranged from 2.7–4.3 liters/minutes in these patients, although values as high as 9 liters/minute have been calculated in other patients not presented here. Total left ventricular outputs ranged from 7.4–8.4 liters/minute at rest. Left ventricular pressure work at rest varied from 6.7–8.2 $kg.m/min/m.^2$ It was observed that *1*) MRA was relatively small, being one-third or less of the area of the mitral valve stenotic orifice; *2*) the left ventricular-left atrial pressure gradient was extremely large and was a major factor in causing considerable regurgitation, even though the area of the regurgitant orifice was small; and *3*) left ventricular output and pressure work were high normal or moderately elevated in mitral insufficiency when associated with mitral stenosis.

Estimation of the residual volume of the right ventricle of the dog by simultaneous injection of T-1824 and brilliant vital red. D. H. LEWIS AND O. W. SHADLE (introduced by G. P. MCCOUCH). *Dept. of Physiol., Univ. of Pennsylvania Med. School, Philadelphia.*

Morphine-Dial-urethane anesthetized dogs were catheterized with two Cournand catheters. Under fluoroscopic guidance the tip of one was placed at the pulmonic valve and the other at the tricuspid valve. Simultaneous instantaneous dye injection was done, one dye in each catheter. Arterial samples from the common carotid artery were collected in tubes placed around the rim of a rotating kymograph drum, allowing approximately 0.67 seconds per tube. Plasma samples were analyzed with a Beckman spectrophotometer using the alcohol precipitation technique described by Dow and Pickring. T-1824 was read at 625 mμ, and BVR at 508 mμ. Correction was made for the absorption of T-1824 at 508 mμ. Time-concentration curves were constructed on semilog paper and from these data cardiac output and mean circulation time were calculated. The difference in mean circulation times of the two dyes is the mean circulation time from catheter tip to catheter tip. When multiplied by the flow this gave the volume of blood between catheters which we interpret as the volume of the right ventricle. Subtracting stroke volume from this gave a figure which may be related to the residual volume of the right ventricle. Residual volumes of from 0–60% of the total right ventricular volume were obtained. Mean circulation time through the ventricle, varying inversely with the residual volume, ranged from 0.5–2.0 seconds. Recirculation of dye did not occur until all or almost all of the primary curve had been obtained.

Activation of dog serum profibrinolysin by a serum fibrinolysokinase. JESSICA H. LEWIS AND JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill.*

Dog serum profibrinolysin may be converted to active fibrinolysin by fibrinolysokinases of different types: *1*) bacterial, e.g. staphylokinase, and *2*) tissue, e.g. purified lung kinase. Certain observations suggest that a third type is present in normal serum. Whole serum treated with chloro-

form loses antifibrinolysin (? also antifibrinolosokinase) and gradually develops active fibrinolysin as the profibrinolysin titer falls. This is shown to be independent of the cell and platelet content of the original plasma. Purified profibrinolysin, treated similarly with chloroform, does not activate, although it is free from antilysin, suggesting that this purified fraction lacks a kinase present in the whole serum. By employing different fractionation technics, various serum fractions, all high in profibrinolysin and free from antilysin, were obtained. Some activated spontaneously, while others did not, again suggesting the presence of a kinase in some fractions. Finally, preliminary observations were presented concerning the preparation of a serum fraction, free from prolysin and antilysin, and containing part of the kinase present in the original serum. This fraction is shown to cause slow activation of purified profibrinolysin.

The α and β lipo-protein pattern of normal and pathological human sera. LENA A. LEWIS, ARDA A. GREEN AND IRVINE H. PAGE. *Cleveland Clinic Research Div. and Frank A. Bunts Educational Inst., Cleveland, Ohio.*

Ultracentrifugal patterns of α and β lipoproteins of human serum have been obtained. A density of 1.21, produced by addition of KBr, was used for preparative concentration and analytical ultracentrifugation. The normal pattern includes the β—lipoprotein and faster moving elements described in previous investigations (GOFMAN, J. W. *Science* 111: 166, 1950) and in addition α—lipoprotein and at least one other component. The normal patterns showed wide variation in the concentration of the different components. The effect of age and sex on the pattern will be discussed. The normal patterns have been compared with those obtained on pathological sera. Also comparisons of the α and β—lipoprotein pattern with the serum electrophoretic pattern will be made.

Equipotential maps of the posterior ectosylvian area and acoustic I and II of the cat during responses and spontaneous activity. JOHN C. LILLY. *E. R. Johnson Fndn., Univ. of Pennsylvania, Philadelphia.*

By means of the *bavatron* (*Am. Inst. Elec. Eng.* 37, March 1940; *Federation Proc.* 9: 78, 1950; *E.E.G. Clin. Neurophysiol.* 2: 358, 1950) relative potentials are recorded from 25 implanted electrodes simultaneously at 7.8 msec. intervals in a region including parts of acoustic I and II (AI and AII) and posterior ectosylvian (Ep of Rose and Woolsey) of a cat's cortex. Equipotential contour maps are made from these data taken at a fairly deep anesthetic level. The two types of lagre 'forms' (responding and 'spontaneous') seen in the activity of this region repeat themselves (with minor variants) in their sites of origin, in their transformations with time, and in their interactions. AI is distinguished from AII by having a quicker die-away of the responding form; AI and AII, from Ep, by response contours which parallel the boundary between AI–AII and Ep. 'Spontacts' (spontaneous activity forms) arise in the middle of Ep (EpII), revealing, also, the AI–AII and Ep boundary. The dorsal end of Ep (EpI) generates forms which are similar during both responses and spontacts. At times with very loud, long (relative to a click) noises, spontacts can be induced in Ep which follow response forms in AI–AII; this effect is masked by a steady background noise. These observations suggest that at least 4 functional areas exist in this region: AI, AII, EpI, and EpII.

Does vagus excitation liberate pyloric gastrin? ROBERT K. S. LIM AND PHILLIP MOZER.* *Dept. of Physiology, Creighton Univ. Med. School, Omaha, Nebr.*

Vagus stimulation (sham feeding in oesophagostomized gastric fistula dogs) evokes remarkably long (2–3 hours) gastric secretory response considering 'strength' (150 gm. meat) and 'duration' (30–90 seconds feeding) of stimulation, suggesting peripheral liberation of chemical agent (gastrin) which outlasts stimulus rather than prolonged central afterdischarge as underlying mechanism. In dogs with pylorus excluded (as vagally innervated pouch) and two fundus pouches (one with, the other without vagal innervation), feeding meat elicits 3-hour HCl response from the former only. The first half of this response is due to vagal stimulation. Application of 5% cocaine to pyloric pouch during first or second 30 minutes following feeding, inhibits subsequent HCl response. The initial 30–60 minutes of uninhibited response results from direct vagal excitation of the fundus, while the first 30–60 minutes of inhibited remainder is attributable to suppression of vagal release of pyloric gastrin. Failure of the vagotomized fundus pouch to respond does not invalidate this conclusion but indicates that the amount of gastrin liberated by the vagus is subthreshold. After oesophagostomy, sham feeding evokes about the same size of response from the innervated fundus pouch, but pyloric cocainization frequently abolishes this response. Apparently, when vagal stimuli to both fundus and pylorus are subliminal for the innervated fundus, they may become effective by summation or potentiation, unless pyloric component is blocked (Uvnäs). If pylorus is vagotomized, the sham feeding response is reduced and pyloric cocainization no longer brings about its total inhibition. Thus vagal stimuli to fundus can excite independently of gastrin.

Effect of DDT on cholinesterase activity of blood plasma of white leghorn cockerels. V. F. LINDEMAN AND HAROLD BURLINGTON. *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

Beginning with the 8th day posthatch, white leghorn cockerels were given daily subcutaneous injections of purified DDT dissolved in chicken fat. The initial dosage was 15 mg/kg. body wt. and was gradually increased until each bird was receiving 225 mg/kg. body wt. at the end of the experiment. Total DDT administered to each bird averaged 2.549 gm. A similar group of birds injected simultaneously with chicken fat were maintained as controls. Weight charts kept for both groups showed that the weights of the control and treated animals paralleled each other. (See BURLINGTON AND LINDEMAN. *Proc. Soc. Exper. Biol. & Med.* 74: 48.) After approximately 85 days of treatment, blood samples were withdrawn and cholinesterase estimations were made on the plasma. The Warburg technique was employed for all experiments; 6 treated and 6 control birds were tested. Experiments were run in triplicate, i.e. 3 manometers containing plasma from control bird and 3 manometers with plasma from treated bird. In all cases except one, the treated animals exhibited a considerably higher cholinesterase activity than the controls. The mean cholinesterase activity of the 6 controls was 13.9 cu. mm. CO_2 per cc. plasma per minute, whereas the treated birds averaged 26.1 cu. mm. CO_2 per cc. plasma per minute. This is almost a 100% increase. Experiments are being conducted in an attempt to explain this marked increase in plasma cholinesterase.

Effect of nitrogen mustard administration upon induced proteinuria in the rat. RICHARD W. LIPPMAN AND HELEN J. UREEN.* *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.*

Because of the reported beneficial effect of nitrogen mustard of the proteinuria in patients with glomerular nephritis, it seemed worthwhile to investigate the effect of nitrogen mustard in another type of proteinuria, caused by the parenteral administration of albumin. Albino rats of the Slonaker strain were rendered proteinuric by the intraperitoneal administration of human serum albumin, under standard conditions, as previously reported. The rate of proteinuria was determined at intervals up to 48 hours by the biuret method. Another group of rats was treated in the same way, but simultaneously with each of the three intraperitoneal injections of human albumin; each rat was given 0.67 mg/kg. methyl-bis(β-chlorethyl) amine hydrochloride by intravenous injection. During the first 7 hours, when very little proteinuria is produced by this method in the control animals, there was no significant difference between the two groups. However, in the group of animals that received nitrogen mustard the mean rate of proteinuria was double the mean rate in the control group at all intervals after the first 7 hours, with no overlapping between the groups.

Effect of atrial systole on closure of the A-V valves. ROBERT C. LITTLE. *Div. of Physiology, Univ. of Tennessee, School of Biological Sciences, Memphis.*

Right atrial and right ventricular pressures were recorded simultaneously with Gregg optical manometers in dogs having an open chest. The effects of atrial systole on ventricular pressure were observed at the beginning, peak and end of atrial systole and at the beginning of ventricular systole (Z point). With normal venous return, the increase in ventricular pressure resulting from atrial contraction was sufficient to reverse the atrial-ventricular pressure gradient during the succeeding atrial relaxation and for a variable interval thereafter. The A-V valves were closed by this slight reversal in gradient before the onset of ventricular systole. This was shown by the failure of ventricular pressure to decrease as atrial pressure did during this presystolic period. Apparently the first heart sound resulted from the sudden tensing of the closed valve by the increased ventricular pressure at the beginning of systole. When the ventricle contracted without a preceding atrial contraction, there was some regurgitation into the atrium during early ventricular systole but this regurgitation normally was absent when atrial contraction preceded the ventrical systole. The regurgitation probably was due to the sudden increase in ventricular pressure which closed the open A-V valves.

Time required for metabolic response to single injections of thyroxin. ROWLAND E. LOGAN* AND ALLEN LEIN. *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Because of conflicting reports concerning the time of appearance of the effects of thyroidal substances, a study was made of the course of the metabolic response during the first 24 hours following the injection of thyroxin. Male guinea pigs weighing between 315 and 455 gm. were used. Measurements of oxygen consumption were made on fasted animals under ether anesthesia in a closed circuit apparatus. The degree of reliability of the technique is indicated by the mean coefficient of variation for 5 untreated animals over a 29-hour period, which was 3.38%. The rate of oxygen consumption of each animal was measured 1 hour before the intraperitoneal injection of 1 mg. of thyroxin, and at 4-hour intervals after the injection. Control animals were injected with dilute sodium hydroxide solution. For each post-

injection measurement the percentage change from the pre-injection value was calculated for each animal. The mean changes for 5 thyroxin-injected animals were: 4 hours, + 5.85% ± 2.86% (S.D.); 8 hours, +14.40% ± 2.89%; 12 hours, +14.75% ± 2.42%; 16 hours, +15.01% ± 3.13%; 20 hours, 16.69% ± 6.29%; 24 hours, +19.60% ± 7.13%. The mean changes for 5 control animals were: 4 hours, −1.24 ± 2.77%; 8 hours, −2.20% ± 3.68%; 12 hours, −0.85 ± 3.91%; 16 hours, −3.38% ± 5.80%; 20 hours, −4.94% ± 4.74%; 24 hours, −2.16% ± 6.48%. The difference between the mean changes of the two groups is highly significant ($P < .01$) as early as 4 hours.

Comparison of cardiac stroke volume as determined by pressure pulse contour method and by a direct method using a rotameter. FRANK H. LONGINO* AND DONALD E. GREGG. *Army Med. Service Graduate School, Washington, D. C.*

Right heart output was measured beat by beat in open-chest dogs by cannulation of the pulmonary artery and use of an optically recording rotameter. Carotid artery pressure pulses were recorded and left heart stroke volumes calculated from them by the pressure pulse contour method of Hamilton and Remington. Comparisons were made in 75 records from 10 dogs. The rotameter was used as standard of reference. Maximum differences between the two methods were +31 and −40% with an average deviation of ±13.1%. It was noted, however, that at diastolic pressures greater than 120 mm. Hg values obtained with the pressure pulse contour method fell below the measured flow (average −25%). If these pulses were eliminated, maximum differences between the two methods became +31 and −28% with an average difference of ±12.0%. Except for this increasing difference at high diastolic pressures, variation between the two methods was not seemingly influenced by heart rate, blood pressure, stroke volume, cardiac output per minute or pattern of the pressure pulse.

Entero-hepatic circulation of bromsulphalein: observations on hepatic, portal and arterial dye concentrations after intra-duodenal and intraportal bromsulphalein administration. STANLEY H. LORBER,* HARRY SHAY AND HERMAN SIPLET.* *Fels Research Inst. and Dept. of Physiology, Temple Univ. School of Medicine, Philadelphia, Pa.*

We previously observed in man that the intraduodenal instillation of bromsulphalein resulted in demonstrable serum dye levels. To confirm this observation and to obtain data relative to its significance in the study of hepatic circulation, the following experiments were performed. Frequent serum dye levels were determined upon specimens obtained simultaneously from the portal vein, hepatic vein, and femoral artery after the intraduodenal instillation of bromsulphalein. Throughout the 45-minute test period significant dye concentrations were present in all specimens. Dye levels were always highest in the portal vein and could be demonstrated a few minutes after dye instillation. No constant relationship was observed between peripheral artery and hepatic vein B.S.P. levels, the variations being due in part to continuous dye absorption. To more clearly demonstrate the relationship of B.S.P. absorption to hepatic vein and peripheral artery dye levels, a single dose was administered through the portal vein. Hepatic vein dye concentrations greatly exceeded arterial levels in the first minute after injection but thereafter hepatic vein levels were usually lower than those observed in portal vein or peripheral artery. An analysis of the data shows that transport of B.S.P. from the gut to the liver and thence to the systemic circulation is rapid. The results suggest that the absolute amount of B.S.P. excreted into the intestine and the resultant entero-hepatic circulation must be considered in evaluating clinical observations employing this dye.

Colorimetric determination of gentisic acid. J. LOWENTHAL (introduced by L. B. JAQUES). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

A solution of gentisic acid (2:5 dihydroxy benzoic acid) is treated in a buffered medium (pH 3.0–4.0) with an excess of ferric chloride and a reaction takes place leading to the formation of ferrous ions. The excess of ferric chloride is rendered colorless by the addition of potassium fluoride. Orthophenanthroline is added and the intensity of the color produced by the formation of the ferrous complex is read within 15–30 minutes after the addition of ferric chloride, the red color having an absorption maximum at 150 mμ. A method for the estimation of gentisic acid in plasma has been worked out.

Changes in concentration of nitrogen during respiratory cycle breathing air. ULRICH C. LUFT, BRUNO BALKE* AND WALTER B. BOOTHBY. *USAF School of Aviation Medicine, Randolph Field, Tex.*

Recent modifications (Rochester, Minn., Lund, Sweden) made on the Nitrogen Meter (LILLY, ANDERSON, HERVEY) have increased the stability and sensitivity of this instrument sufficiently to justify attempts to measure continuously the changes in nitrogen concentration during the respiratory cycle while breathing air. In preliminary tests it was found that fluctuations in water vapor content affected the measurements con-

siderably in the range of nitrogen in question. For this reason the inspired air was humidified at body temperature before it entered the respiratory valve from which a continuous sample was bled close to the mouthpiece. Using a recording device of adequate frequency-response differences between the inspired and expired nitrogen fraction were obtained with simultaneous tracings of tidal and total respired volume. The characteristic pattern observed in the steady resting state, where the expired nitrogen is higher than the inspired fraction, changes immediately with a few forced respirations. The expired nitrogen fraction then falls below that of inspired air, returning to the initial level in the course of minutes. Recordings taken after breathholding, at different barometric pressures and in various types of exercise demonstrate the applications of this method to discern rapid respiratory and metabolic changes which are reflected in the expired nitrogen concentration.

Hemolysis by freezing. BASILE LUYET AND LEO MENZ.* *Dept. of Biology, St. Louis Univ., St. Louis, Mo.*

While freezing is one of the classical means of producing hemolysis, red cells may, under certain conditions, remain intact in frozen blood. To study some of these conditions we determined, by means of the hematocrit, the proportion of intact cells in oxalated ox blood which was: *1*) frozen by immersion of one cc. quantities in baths at various temperatures from −3° to −8°C., *2*) kept in the frozen state at these temperatures for various lengths of time, and *3*) thawed at room temperature. The temperature within the blood was measured with a thermocouple. A comparison of survival curves, freezing curves (temperature/time) and ice curves (quantities of ice formed in given time at various temperatures; cf. LUYET AND SCHMIDT. *Federation Proc. 1950*) shows that: *1*) Hardly any hemolysis occurs when all the water of the blood which can freeze at −3° is frozen. *2*) The degree of hemolysis increases gradually with decreasing bath temperatures when most of the freezable water has been given time to freeze and the temperature of the frozen blood has been allowed to reach the temperature of the bath; (there were about 90% intact cells after a 30-minute congelation at a bath temperature of −4°; and some 15% after a 10-minute congelation at −8°). *3*) At all the temperatures between −4° and −8° the percentage of hemolysed cells increased only a few units when the blood remained frozen for 2 hours. Thus hemolysis seems to occur when the last fraction of freezable water freezes. The mechanism of that destructive action of congelation still remains to be determined.

Some properties of cation exchange resins, with reference to their clinical applications. EVAN W. MCCHESNEY. *Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

In vitro experiments involving the shaking of cation exchange resins with buffer solutions of pH 6.4, for 3 hours, indicate that the effective capacity of the best available sulfonic resins is about 3.5 mEq/gm., and that of the best carboxylic resin is about 2 mEq/gm. The various resins differ considerably in their relative affinities for sodium and potassium. In artificial gastric juice the sulfonic resins give up about 30% of their alkali for H, and an approximately equal amount in exchange for potassium and/or sodium. The carboxylic resin gives up to gastric juice most of the alkali it holds, all in exchange for H. Feeding experiments with rats indicate that the sulfonic resin tested has a higher effective capacity than does the carboxylic. Both types of resin may be administered partly in the K cycle. This provides a positive K balance, reduces the metabolic acidifying effect of the resin, and does not drastically reduce its sodium-binding power, providing that the K-form resin does not exceed one-third to one-fourth of the resin intake.

Comparative study of complex breathing cycles in terrestrial mammals. F. H. MCCUTCHEON (introduced by RAYMOND W. ROOT). *School of Veterinary Medicine, Univ. of Pennsylvania, Philadelphia.*

Detailed study of physical factors related to breathing in the horse and rat showed processes which are superficially quite different in gross pattern. A polyphasic cycle is described for the horse, which is in contrast to a diphasic cycle complemented by a rhythmically recurring accessory cycle for the rat. The complementary cycle is shown to be typical for other mammals (guinea pig, mouse, rabbit, cat, dog, man) with decreasing frequency related to slower breathing rates. Analysis of the complementary cycles of rats seems to indicate that an intra-pulmonary retention and compression of air occur. Similar compression could be inferred from correlation of factors in horse breathing, and it is suggested that an independent regulation of pressure at respiratory membranes is a fundamental similarity for mammalian breathing. Gross difference in breathing processes are thus explained at the common level of diffusion regulation.

Human glomerular permeability and tubular recovery values for hemoglobin. ROGER K. MCDONALD, JOHN H. MILLER* AND ELEANORE B. ROACH.* *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and Section of Gerontology, Baltimore City Hospitals, Baltimore, Md.*

The mechanism of hemoglobin excretion by the human kidney has been studied in 19 subjects. From the data it is concluded that hemoglobin is filtered by the glomeruli and recovered to a limited

extent by the tubules. The permeability of the glomeruli to hemoglobin averaged 12.9% of the glomerular permeability to inulin. The tubular recovery of hemoglobin averaged 17.1 mg/100 cc. of glomerular filtrate. The values for both glomerular permeability and tubular recovery were significantly lower when the hemoglobin was administered by slow infusion than when the total amount of hemoglobin was injected over a period of 5–10 minutes. The present values for glomerular permeability to hemoglobin are considerably higher than those which apparently obtain for plasma albumin. Presumably the present values for glomerular permeability to hemoglobin reflect an alteration in the membrane permeability induced by the administration of hemoglobin. How this effect is mediated is not known, but several possible mechanisms will be discussed.

Influence of acute emotions on blood clotting. DAVID I. MACHT. *Dept. of Pharmacology, Sinai Hosp., Baltimore, Md.*

Studies on rabbits and cats before and after enraging them showed that the clotting of whole blood studied by the Lee and White method was enormously shortened after rage and profound terror. The sera of these animals were also markedly phytotoxic when studied by Macht's phytopharmacological methods (*Bull. Torrey Botan Club.* 76: 235, 1949). The phytotoxicity, however, was retained for 2 and 3 days, thus indicating that increased secretion of epinephrine is not completely responsible for the phenomena observed. In the present investigation, clotting time of blood by the Lee and White method was studied on healthy persons in the blood bank department of Sinai Hospital. The individuals were classified into three groups: *1*) persons apparently normal and composed; *2*) persons apprehensive or mildly nervous; *3*) persons highly nervous and worried. Approximately 400 experiments have so far been performed. It was found that normal non-emotional individuals gave an average clotting time of 8.3 minutes; those of the apprehensive group, an average clotting time of 3.4 minutes; and those who were highly nervous or hysterical, an average clotting time of 2.2 minutes. In most of the individuals who were very nervous the blood pressure was also considerably elevated. In these clinical cases no difference in phytotoxicity was observed between the three groups.

Subcortical localization of certain 'taste' responses in the cat. MARTIN B. MACHT. *Dept. of Pharmacology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.*

In the course of an investigation of the behavioral capacities of long-surviving mesencephalic and bulbospinal cats it was observed that they would accept certain substances as food but would reject others. The normal diet of these animals consists of weighed amounts of hog liver. Mesencephalic and bulbospinal preparations readily swallow liver cubes dipped in a 0.9% solution of sodium chloride, if the cubes are placed on the tongue. In all such animals deglutition is followed by chop-licking. It was noted that mesencephalic cats spat out liver cubes dipped in quinine sulfate. This observation led to a more complete investigation of the acceptance or rejection of bitter, salty, sour, and sweet substances by these animals. The solutions used as test agents were quinine sulfate, sodium chloride, acetic acid and dextrose. Fourteen cats were studied in a series of 84 experiments. The dorsal level of brainstem transection in each instance was at the rostral border of the superior colliculi. The ventral level, however, varied from a point just behind the mammillary bodies to one at the rostral border of the pons. Dextrose-dipped cubes were never rejected by any animal regardless of the concentration of the dextrose. Cubes dipped in quinine, saline or acetic acid solutions were rejected by all animals at certain concentrations. Thresholds for rejection were determined and were found to be essentially the same as those at which the substances were rejected by normal cats. Threshold concentration for rejection is believed to be appreciably lower than the concentration necessary for the production of pain. It is concluded that certain discriminatory responses relating to 'taste' have a subcortical localization in the cat. Motion pictures of these responses have been filmed.

Direct pulmonary effects of pressure breathing. JAMES V. MALONEY, JR.* AND JAMES L. WHITTENBERGER. *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

The capacity and resistance of the pulmonary vascular bed and the effects of airway pressure on the alteration of fluid exchange in the lung have been of considerable interest in physiology and clinical medicine. One phase of the problem is concerned with the effects of normal breathing and positive pressure breathing on the relationships between intrapleural pressure, alveolar pressure, and pulmonary 'capillary' pressure. In a series of dog experiments these pressures were compared during spontaneous breathing and intermittent positive pressure breathing. Airway pressure and pulmonary 'capillary' pressure were then plotted relative to simultaneous intrapleural pressure. Results will be presented in the form of graphs. Two factors of major importance became apparent: *1*) the effects of alteration of venous return brought about by changes of pressure relationships in the venous system within and without the thorax, and *2*) the effects of lung volume change.

At a given lung volume, intrapleural pressure, alveolar pressure, and pulmonary 'capillary' pressure bore the same relationship to each other regardless of the method used to inflate the lung. It was concluded that the hemodynamic effects of pressure breathing are due to effects on venous return, and not to any direct effect on the lungs other than volume change.

Attempts to produce shunting of blood from canine and leporine renal cortex. N. S. R. MALUF. *Dept. of Pharmacology, Univ. of Louisville School of Medicine, and Surgical Research Unit, Brooke General Hospital, Louisville, Ky.*

The canine vesical trigone was exteriorized (*Ann. Surg.* 130: 43, 1949). The exposed sciatic nerve of dogs under light Nembutal was stimulated for about 20 minutes by rapid inductorium shocks, making the dog hyperpneic. The rates of glomerular filtration and effective renal blood flow tended to be reduced. Intra-aortic injection of India ink showed, however, no evidence for shunting of blood from the renal cortex. These injections were performed in almost bloodlessly laparotomized animals. Large intravenous doses of epinephrine (0.22 mg/kg.), crushing tourniquets to a thigh for 5 or more hours under light barbital, histamine-shock (5 mg/kg. histamine; 2 HCl in cotton-seed oil and lanolin subcutaneously) and hemorrhage (rapid removal of over ⅓ of the total blood volume under light ether) did not elicit shunting of blood from the renal cortex to the medulla in the dog. In the shock states there was merely diminution of blood flow throughout the kidney. Injecting a relatively large amount of sterile, fresh, human blood (15 cc/kg.) slowly intravenously into rabbits did not result in renal cortical ischemia either immediately after the injection or close to the fatal end, within 3 days later. If the injection were rapid the rabbits convulsed and died immediately, presumably due to rapid release of potassium from lysed human erythrocytes. Rapid injection of an equivalent amount of the citrate-dextrose anticoagulant was harmless.

Cerebral blood flow and oxygen consumption during natural sleep. R. MANGOLD,* L. SOKOLOFF,* P. O. THERMAN, E. H. CONNER,* J. I. KLEINERMAN* AND S. S. KETY. *Dept. of Physiology and Pharmacology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.*

By means of the nitrous oxide method cerebral blood flow (*CBF*) and cerebral metabolic rate in terms of oxygen consumption (CMR_{O_2}) were measured during natural sleep in 5 healthy young men. Sleep was favored by forced wakefulness for approximately 20 hours. Each study consisted of a determination before and another during sleep. The conditions during both measurements were identical. Continuous EEG recordings served as the chief criteria for the evaluation of the mental state. On 13 subjects unable to fall asleep duplicate measurements were obtained under the same conditions as a control of variations found in two consecutive studies. In these 13 control studies the CBF fell from a mean of 59.0 cc/100g/min. to a mean of 55.6 cc/100g/min. and the CMR_{O_2} from a mean of 3.50 cc/100g/min. to a mean of 3.30 cc/100g/min. Neither of these differences was found to be statistically significant ($P_{CBF} > 0.2$; $P_{CMR} > 0.2$). During sleep, a slight increase in CBF was observed (mean control 59.6 cc/100g/min., mean sleep 64.6 cc/100g/min.) to a degree approaching significance compared to the duplicate controls (P approx. 0.15). CMR_{O_2} during natural sleep shows a slight decrease (mean 3.20 cc/100g/min. compared to mean 3.42 cc/100g/min. during wakefulness) which, however, on statistical analysis was identical with the decrease observed on duplicate controls. On the basis of this limited series, no significant difference in metabolic activity of the brain tissue during sleep and wakefulness could be detected, whereas the CBF appears to rise slightly during sleep.

Functional studies of the anterior tibial muscle. J. E. MARKEE AND M. WILLIAMS. *Dept. of Anatomy, Duke Univ., Durham, N. C.*

The anterior tibial muscle is especially subject to paralysis. It has multiple origins, insertions, functions and innervations. In the human, 5 nerve branches are distributed to this muscle. In the dog, two different techniques demonstrated that shortening occurred in the separately innervated areas of this muscle. *1*) Markers were placed on the surface of the muscle and photographs are made before and during stimulation of the individual nerve branches. The amount of shortening in a given area was determined by measuring the difference in the distance between the dots in a given area during the control period and during stimulation. *2*) The areas which contract during the electrical stimulation of each branch were mapped electrically by recording the action potential from the surface and the depth of all of the areas of the muscle. The 5 separate nerve branches produce contraction in each of 5 separate areas which do not overlap. Certain of these areas act in tandem since contraction of some of the combinations of areas produces more shortening without significantly greater tension. Conversely other areas of the muscle act in parallel since contraction of these combinations produces increased tension without producing more shortening than resulted from contraction of the individual areas. Perhaps the arrangement of part of these separate func-

tional areas of the muscle in series and part in parallel is related to the multiple functions of this muscle and to the fact that it is especially susceptible to paralysis.

ACTH on experimental proteinuria in the rat. J. MARMORSTON, A. SELLERS, H. GOODMAN AND S. SMITH, III (introduced by HARRY GOLDBLATT). *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.*

We have previously reported that the normal rat responds to the intraperitoneal injection of hog renin by an intense transient proteinuria in the hour following renin injection. It has further been reported that bilateral adrenalectomy abolishes this proteinuria and that the proteinuria response to renin in the salt-maintained adrenalectomized rat is restored by the injection of suitable doses of cortisone, desoxycorticosterone acetate, or aqueous adrenal cortex extract. ACTH in doses equivalent to 0.4 mg. of Armour's Standard was injected subcutaneously into normal rats at 12 noon and 5 P.M. of *day 1* and at 10 A.M. *day 2*. Half of the experimental animals and all of the controls received 4 Goldblatt DU of hog renin in 4 cc. of physiological saline intraperitoneally at 11 A.M. The remainder of the experimental animals received a similar injection of renin at 1 P.M. Urines were collected from all animals during the 60 minutes following the renin injection. The prior administration of ACTH in the dose and schedule indicated caused a marked increase in renin proteinuria when renin was given one hour following the last ACTH injection. When 3 hours were allowed to elapse following the last ACTH injection no increase in renin proteinuria was found. Control animals received saline injections instead of ACTH.

	NO. OF RATS	URINARY PROTEIN, MG/HOUR
Control	17	47.8
ACTH-Renin interval 1 hour	25	74.5
ACTH-Renin inteval 3 hours	27	42.1

Acute renal disease consequent on combined treatment with desoxycorticosterone, sodium chloride and renin. G. M. C. MASSON, A. C. CORCORAN AND I. H. PAGE. *Cleveland Clinic Fndn. and Frank E. Bunts Educational Inst., Cleveland, Ohio.*

We have previously described a syndrome, reminiscent of eclampsia, consisting of edema, oliguria and convulsions which can be elicited by subcutaneous injection of renin into DCA-hypertensive rats. The present report deals with more detailed studies. It was found that administration of renin at the outset of treatment with DCA and saline (1%) does not elicit the disease; instead of oliguria we noted an increased diuresis resulting from the additive diuretic effects of renin and DCA. Rats in which hypertension has been established by treatment with 2% NaCl without DCA develop the syndrome in mild form, while rats on 1% saline alone show only occasional evidence of the disease. The syndrome can most easily be induced by injecting renin into rats already made hypertensive by means of both DCA and salt. Azotemia and increased clotting time occur. The urine is scant, proteinuric and urea clearance is usually low. Gross pathologic findings are anasarca, hemorrhages in the endocardium, intestine, bladder and brain; renal microscopic lesions, in addition to those pre-existing from treatment with DCA and saline are hemorrhages into the tubules and accumulation of pigment casts with tubular necrosis. Thus the histological picture resembles that of experimental crush syndrome. Pituitrin, thromboplastin, chymotrypsin, and trypsin, unlike renin, did not precipitate the syndrome. The condition is of interest insofar as it may relate in its pathogenesis to toxemia of pregnancy on one hand and to crush syndrome (lower nephron nephrosis) on the other.

Antithyrotoxic action of antibiotics in rats. JOSEPH MEITES, R. C. OGLE* AND Y. S. L. FENG.* *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Vitamin B_{12} has been shown to be very effective in counteracting growth-retardation in rats induced by administering large doses of thyroid-active materials. Since certain antibiotics also promote growth when fed in small amounts, it was of interest to see whether they were likewise effective in overcoming thyrotoxicosis. Forty young female albino rats were divided into 4 uniform groups, and were fed a basal ration which contained 20% whole milk powder as the sole source of vitamin B_{12}. The following substances were added to the ration of each group and fed for 36 days: *1*) controls, nothing; *2*) 0.16% thyroprotein; *3*) 0.16% thyroprotein and 0.05% procaine penicillin G (Merck); *4*) 0.16% thyroprotein and 0.05% neomycin sulfate (Upjohn). The total average gain in body weight and daily average food intake in grams per rat were as follows for each group: *1*) 36.5 and 10.94; *2*) 24.3 and 13.68; *3*) 41.0 and 15.24; *4*) 50.5 and 15.3. Basal metabolic rates were determined at the end of the experiment, and showed that thyroprotein alone (*group 2*) increased oxygen consumption 74% above the controls (*group 1*). Neither antibiotic counteracted the thyroprotein-induced rise in metabolism, indicating that the former did not increase elimination of the latter from the body. At least part of the effectiveness of the antibiotics in these experiments can be attributed to an increase in daily food con-

sumption and to a greater efficiency in converting food into body weight gains.

Counteraction of anabolic effects of mild hyperthyroidism by vitamin B_{12}. JOSEPH MEITES. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Vitamin B_{12} has been shown to counteract the growth-retarding effects induced in rats by administering thyroid-active materials. Young mice, unlike rats, respond to the administration of low doses of thyroid substances by true increases in growth rate, and it was of interest to determine whether vitamin B_{12} could modify this effect. Forty immature albino mice (Rockland), weighing approximately 13.5 gm. each, were divided into 4 uniform groups and fed a basal ration low in vitamin B_{12} for 30 days. The following substances were added to the ration of each group: *1*) controls, nothing; *2*) 0.025% thyroprotein (Cerophyl); *3*) 100 μg. of vitamin B_{12} (Merck) per kg. of ration; *4*) 0.025% thyroprotein and 100 μg. of vitamin B_{12}. The total average gain in body weight and daily average food consumption in grams per mouse were as follows for each group: *1*) 9.6 and 3.2; *2*) 18.5 and 5.4; *3*) 15.9 and 4.0; *4*) 10.6 and 4.0. It can be seen that either thyroprotein or vitamin B_{12} alone considerably increased body weight and food intake above the controls, while the combination of the two substances eliminated the favorable effects of either on body growth. The efficiency in converting food into body gains was greatest for the groups receiving thyroprotein or vitamin B_{12} alone, and least for the group receiving both substances.

Cortisone and mechanism of increased capillary permeability in inflammation. VALY MENKIN. *Agnes Barr Chase Fndn. for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Pa.*

Leukotaxine is of significance in the mechanism of the initial increase in capillary permeability with the development of inflammation. It is conceivable that there may be other substances concerned in the mechanism (*Dynamics of Inflammation*, 1940). Adrenal cortex extract and cortisone inhibit the increase in capillary permeability induced by an exudate or leukotaxine (*Am. J. Physiol.* 129: 691, 1940; *Proc. Soc. Exper. Biol. & Med.* 51: 39, 1942). Present studies indicate that the adrenal cortex extract or cortisone inhibit the increase in capillary permeability caused by an alkaline exudate. But when an acid exudate is injected locally cortisone or the whole adrenal extract do not seem to inhibit the accumulation of trypan blue from the circulation. The evidence points to the presence of another factor in acid exudates not present in alkaline exudate and which causes a marked increase in capillary permeability. The reaction seems more pronounced than that induced by the diffusible leukotaxine of alkaline exudates. This factor can be recovered in the pseudoglobulin-albumin fraction of acid exudates. Several precipitations with saturated ammonium sulfate tend to purify this indiffusible factor. This permeability factor seems to serve as a sort of homeostatic mechanism whereby the increased capillary permeability of the initial alkaline pH of an inflamed area is also maintained when it becomes acid in character. The term 'exudin' is proposed for this factor to distinguish it from leukotaxine.

Sodium flux through fish gills. DALLAS K. MEYER (introduced by M. M. ELLIS). *Dept. of Physiology and Pharmacology, Univ. of Missouri Med. School, Columbia.*

Studies on the absorption of Na and Cl against a concentration gradient by the gills of goldfish showed that the two ions, in approximately equivalent amounts, are lost from the fish during the first hour (MEYER. *Science* 108: 305, 1948). This loss is apparently through the gills, for the urinary NaCl was collected separately and there was no significant regurgitation of intestinal contents. In order to determine whether this phenomenon was due to an increased permeability of the gills, an inhibition of the transport mechanism, or a combination of both factors, a series of studies employing Na^{22} was made. The fish were placed in 1 mM. NaCl containing Na^{22} and the net Na concentration and Na^{22} followed for 12 hours. The total concentration of Na in the surrounding medium increased an average of 20% at the end of 1 hour and then decreased for the next 11 hours as active Na uptake became evident, although the Na^{22} decreased at a steady rate over the entire 12-hour period. A second series in which Na^{22} was injected into the fish by intracardiac puncture demonstrated a moderate loss of Na^{22} from the gills throughout the 12 hours, but the rate of loss was greatest during the first hour. Sodium ion absorption by the fish gill proceeds at a steady rate which normally more than replaces the loss through the gills. However, under stress there is an increase in the outward movement of Na presumably due to an increase in the permeability of the gill so that the Na loss exceeds the capacity of the uptake mechanism.

Urine volume, a measure of renal function. ALEXANDER J. MICHIE AND CATHARINE R. MICHIE (introduced by J. E. RHOADS). *Dept. of Physiology, New York Univ. College of Medicine, New York City and Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Further exploitation of the differential clearance method developed by Goldring and Chasis showed that in more than 70% of 140 determinations at concentration of inulin or *p*-aminohippurate in any simultaneously collected specimen was practically identical for two kidneys in the same subject. This remained true during and following the administration of adrenaline intravenously despite the changed minute volume of urine flow. It also occurs in unilateral kidney disease if the tip of the ureteral catheter is placed distal to the lesion. Since the urine concentration (U) remains constant and since we maintain a constant plasma concentration (P), the urinary output (UV) and the clearance (UV/P) for each kidney vary directly with the minute urine volume (V). It follows that the ratio of the urinary volume per minute, right to left kidney, is equal to the right to left ratio of the inulin and *p*-aminohippurate clearances and also of the maximal tubular secretion of *p*-aminohippurate. These data add further support to the thesis that rarely is a non-functioning tubule attached to a normally functioning glomerulus or the converse.

Cortical projection of postural impulses. WALTER A. MICKLE* AND HARLOW W. ADES. *Dept. of Anatomy, Emory Univ., Emory University, Ga.*

Studies of the cortical projection of auditory, vestibular, proprioceptive and tactile systems in cats have been made, using the evoked potential technique. Anesthetized animals were stimulated either by physiological means, when practicable, or with short electrical pulses at various points within these systems. Click stimulation of the auditory system has demonstrated that cortical response occurs outside the usually recognized acoustic areas, appearing as a separate area over a part of inferior portions of the anterior ectosylvian and anterior suprasylvian gyri. Electrical stimulation of the vestibular branch of the VIII cranial nerve yields a contralateral field of cortical responses which lies in the same general area as the anterior auditory response. These impulses are apparently carried by fibers in the medial lemniscus. Muscle twitches and stimulation at various positions within the proprioceptive pathways activate two cortical fields, one which overlaps those of the auditory and vestibular projections. In addition, certain tactile stimuli have been shown to have projections within this composite area. The demonstration of a polysensory projection area, responsive to auditory, vestibular, proprioceptive and tactile stimulation, and having the characteristics of a primary projection, suggests that it may represent a field in which integration of stimuli for maintenance of spatial orientation occurs.

Relationship between polarographic protein wave height and total potential sulphydryl content of protein. G. J. MILLAR (introduced by L. B. JAQUES). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

The heights of both waves I and II of the polarograms of crystalline chymotrypsin, chymotrypsinogen, pepsin, egg albumin and insulin are quite constant between electrolyte pH values of about 3.5–8.5 in 1 molar ammonia buffer with 0.001 M cobaltic hexammine chloride. The wave heights vary from protein to protein, however, and seem to be directly proportional to the total potential sulphydryl content of each protein, i.e. the cysteine plus 2 x cystine content expressed as 10^{-5} gm. moles/gm. protein. The implications of this relationship and the effect of ultra violet irradiation of the protein on the relationship will be discussed.

Effects of temperature upon resistance of guinea pigs to anoxia. JAMES A. MILLER, JR., FAITH S. MILLER AND CAROLYN B. FARRAR (introduced by STEPHEN W. GRAY). *Dept. of Anatomy, Emory Univ. School of Dentistry, Emory University, Ga.*

Since the oxygen requirements of living cells vary with temperature, the rationale for the standard practice of warming asphyxiated infants is questioned. In previous studies (Miller '49a, '49b) moderate cooling increased resistance of guinea pigs to asphyxia by 95% N_2 + 5% CO_2. Present investigations cover the range from lethal heat to lethal cold. Cooling was by immersion in ice water, warming by exposure to radiant heat. The time of death of 202 asphyxiated neonatal animals was recorded at colonic temperatures between 45.2°C. and 10.9°C. Deaths from temperature occurred above 44°C. and below 19°C. A linear increase in survival of approximately 50%/10°C. decrease in temperature was recorded for the entire non-lethal temperature range (coefficient of correlation −.8). The shortest anoxic survival was 81 seconds at 44.2°C., the longest 617 seconds at 14°C. Young adults (103) tested similarly showed a linear increase of 35%/10°C. decrease in temperature (coefficient of correlation −.7). Deaths before experimentation occurred above 44°C. and below 16°C. The shortest survival was 57 seconds at 43.5°C., the longest 286 seconds at 15.4°C. Narcosis with sodium pentobarbital (Nembutal) increased the resistance to anoxia at each temperature tested. When tested in pairs, the cooler animals recovered from exposures lethal to warmer litter mates. These experiments show that each degree of temperature reduction increases resistance to asphyxia until cold becomes lethal. (Supported in part by a grant from the Natl. Insts. of Health.)

Effects of parathyroidectomy, ergostanyl acetate and the antistiffness factor on nutritional muscular dystrophy in the rabbit. ANNE E. MILMAN,* A. T. MILHORAT AND JULIA B. MACKENZIE.* *Depts. of Psychiatry and Medicine, Cornell Univ. Med. College, New York Hosp., New York City.*

Nutritional muscular dystrophy, produced experimentally in rabbits by means of the vitamin E deficient diet of Goettsch and Pappenheimer, has been shown to be cured or prevented by the administration of α-tocopherol and several of its derivatives. Nutritional muscular dystrophy which arises in guinea pigs maintained on the diet of Oleson is accompanied by the same symptoms as those seen in the rabbit; namely, by similar histological muscular lesions, by what has been described as stiffness of the leg joints but which is, in reality, a partial immobilization due to shortening of the muscle-tendon unit, and by early death of the animals. These changes may be modified or prevented in the guinea pig by the administration of the antistiffness factor, and of ergostanyl acetate respectively. Therefore both substances have been assayed in dystrophic rabbits, and both have been found to modify the course of the disease and to lengthen the life span of the animal. A disturbance in the metabolism of calcium and phosphorous occurs in experimental muscular dystrophy which is similar to that seen in hyperparathyroidism. A study has been made of the effect of thyroid-parathyroidectomy, of thiourea administration, and of cortisone on animals with muscular dystrophy. The results suggest that the secondary symptoms of dystrophy are alleviated by parathyroidectomy but the basic disturbance is not affected.

Production of anemia in dogs with methylene blue. R. A. MITCHELL* AND H. C. STRUCK. *Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Nebr.*

Anemia produced in dogs by methylene blue has been studied in an attempt to determine the mechanism. Six dogs, after normal hematological levels had been established, were given methylene blue, 3 dogs receiving daily intravenous doses of 10 mg/kg., and 3 receiving 40 mg/kg/day orally. The red cell count in all dogs dropped to about 4 million in 2 weeks. All dogs were then given oral doses of 100 mg/kg/day in divided doses. This caused the red count to drop to between 1.5–3.0 million. The dogs were maintained at this degree of anemia for 3 months. The anemia was proportional to dosage, and withdrawal of the drug resulted in rapid recovery. Hemoglobin was between 3 and 6 gm/100 cc. The erythrocytes were of normal size and shape but with pronounced polychromatophilia, with many nucleated red cells in the peripheral blood. The reticulocyte count varied between 10–25%, depending on the degree of anemia. The white count remained within normal limits in number and distribution, and only in the animals with the severest anemia was there a slight shift to the left. The cell fragility, icterus index, direct and indirect van den Bergh were all normal throughout the period of observation. Bone marrow studies have shown hyperplasia of the erythroid elements with a predominance of normoblasts. The dogs remained in good health and showed no weight loss. There was no jaundice or discoloration of the skin from methylene blue other than soilage from excreta.

Species differences in effects of progesterone-testosterone propionate combinations on reproductive tracts of castrate female opossum and albino rat. CHARLES F. MORGAN. *Dept. of Physiology, Georgetown Med. School, Washington, D. C.*

Progesterone administered alone in 1.0-mg. daily doses for 12 days to the castrate female rat results in an increase in the weight of the uterus and fallopian tubes as compared to the untreated castrate; whereas progesterone administered in 1-mg. and 5-mg. daily doses for 12 days to the castrate female opossum results in a decrease in the weight of the reproductive tract as compared to the untreated castrate (MORGAN, C. F. *J. Clin. Endocrinol.* 6: 468, 1946). T-propionate administered alone in 0.25–1.0 mg. daily doses for 12 days stimulates the uterus of the rat and daily doses of 5 mg. stimulate the uterus of the opossum. Simultaneous administration of progesterone and T-propionate each in doses of 0.25–1.0 mg. per day for 12 days to castrate female rats results in an increase in weight but no greater than that produced by T-propionate alone. In the opossum, progesterone-T-propionate combinations each in 1-mg. doses result in a synergistic action, with the Mullerian duct derivatives weighing four times those of the untreated castrate, and eight times those of the castrate treated with the same dosages of progesterone alone. In summary: *1*) In the opossum, progesterone alone causes regression of the reproductive tract; whereas in the rat, progesterone has a stimulating effect; *2*) T-propionate stimulates the reproductive tracts of both species; *3*) progesterone and T-propionate synergize in the opossum but not in the rat; *4*) in the opossum, progesterone apparently enhances the action of the androgens.

Temperature and metabolism in some Wisconsin mammals. PETER R. MORRISON AND FRED A. RYSER (introduced by C. N. WOOLSEY). *Depts.*

of Zoology and Physiology, Univ. of Wisconsin, Madison.

This study aimed to define the energy cost of temperature regulation in the cold for a number of small indigenous mammals. Metabolism was measured as oxygen consumption automatically recorded from a closed circuit system during contiguous periods of 5–15 minutes. Typical runs were composed of five 2- to 3-hour periods each at a different temperature ranging from 0°–30°C. Measurements on 13 species comprising more than 10,000 individual measured periods were obtained in some 250 runs. Minimum values in generally quiet periods were chosen to represent muscular inactivity. When plotted against ambient temperature these values gave a linear relationship within the limits of variability. This could be reasonably well extrapolated to the body temperature at the abscissa in most cases. This implies that insulation was not changing and that a single constant, the slope of the line, defined heat loss per unit gradient at all ambient temperatures studied. The following average values for species expressed as cc.O_2/gm/hr/°C were obtained: Mus (17 gm.),. 0290; Peromyscus (20 gm.), 0.218; Clethrionomys (24 gm.), 0.226; Zapus (25 gm.), 0.202; Blarina (30 gm.), 0.220; Eutamias (47 gm.), 0.166; Microtus (51 gm.), 0.139; Mustella (64 gm.), 0.152; Glaucomys (75 gm.), 0.090; Scalopus (100 gm.), 0.085; Rattus (170 gm.), 0.092; Citellus (195 gm.), 0.094; and Citellus (460 gm.), 0.047. Values in cc.-O_2/gm$^{2/3}$/hr/°C., *i.e.* roughly corrected to equal surface area, were as follows respectively: 0.738; 0.592; 0.652; 0.590; 0.684; 0.599; 0.516; 0.600; 0.380; 0.395; 0.510; 0.428; and 0.363. The downward trend in this series could reflect the better insulating ability of the longer fur in the larger animals.

Effect of experimental renal hypertension on experimental cholesterol atherosclerosis. W. G. Moss,* J. P. Kiely,* J. B. Neville,* J. E. Bourque* and G. E. Wakerlin. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

To clarify the controversial role of hypertension in the genesis and progress of arteriosclerosis, 11 pairs of dogs, one normotensive and one hypertensive, were placed on the atherosclerosis producing diet of Steiner and Kendall. These pairs of dogs were killed after 2½–9½ months. The degree of atherosclerosis was graded 0 for no lesions to 5 plus for numerous and severe lesions. Nine normotensive dogs were graded 0; one, 1 plus; and one, 4 plus. The last dog showed the highest average normotensive level (140 mm. Hg mean femoral pressure). Three hypertensive dogs graded 0–1 plus had poor hypertensions (130–140 mm. Hg). The remaining 8 hypertensive dogs were graded 2 plus to 5 plus and showed hypertensions from 130–280 mm. Hg. There was some correlation between the hypercholesterolemia and the severity of the atherosclerosis. In 6 of 9 dogs graded 2-5 plus, hypercholesterolemia decreased during the period when atherosclerotic lesions were developing. In 11 dogs graded 0, hypercholesterolemia continued elevated. Four of the 6 dogs graded 4 and 5 plus had numerous atherosclerotic lesions in their renal arteries and showed sharp increases in their hypertensions one or more months before sacrifice. This further narrowing of the renal arteries probably caused the exacerbation in the hypertensions. The severity of the coronary atherosclerosis in the 6 dogs graded 4 and 5 plus suggested the possibility of producing experimental chronic myocardial insufficiency.

Studies of residual air volumes at rest and during treadmill exercise. Hurley L. Motley and Joseph F. Tomashefski.* *Cardio-Respiratory Lab., Barton Memorial Div., Jefferson Hosp., and Dept. of Medicine, Jefferson Med. College, Philadelphia, Pa.*

The residual air volume obtained during treadmill exercise (rate 2 mph, flat) with the oxygen open circuit method was compared with the resting supine measurement in 34 cases of pulmonary fibrosis and/or emphysema. A mean average increase of 0.6% in residual air percentage of total lung volume (TLV) with a S.D. ± 6.24% was observed. In 15 cases with the average resting residual air 44.8% of TLV (all above 35%) the increase with exercise was 1.4%, and in 19 cases with the average resting residual air 29.4% of TLV (all below 35%) the increase with exercise was 0.3%. In 6 cases with extensive emphysema and exhibiting a decrease in arterial oxygen saturation of 2.7% with step-up exercise, the exercise residual air volume was increased 6.8% of TLV, while in 8 other cases with a similar degree of emphysema, but showing an increase of 0.75% in arterial oxygen saturation with step-up exercise, the exercise residual air was decreased 9.2% of TLV. No correlation was noted in the non-emphysema group for the exercise residual air percentage of TLV with arterial O_2 saturation. No apparent correlation was noted for changes in the exercise residual air volume with maximal breathing capacity, vital capacity, or the over-all evaluation of pulmonary function impairment. Changes in the residual air percentage of TLV with moderate exercise appear to be of small magnitude, even in extensive pulmonary insufficiency.

Effects of experimental coronary occlusion on phosphate fractions of the dog heart. A. G. Mulder, William J. Marshall,* Thomas C. Lindman* and Charles D. Proctor.* *Dept.*

of Physiology, Stritch School of Medicine, Loyola Univ., Chicago, Ill.

In these experiments the anterior descending branch of the left coronary artery of the dog heart was ligated. After a variable period of time, usually, 2 hours, the whole heart was rapidly removed (about 5 seconds) and immersed in an ether dry ice mixture. The portion of the heart suffering from the infarction was cut out for analysis. A portion of the non-infarcted left ventricle was also taken for analysis. These results were compared with the results obtained in other normal dog hearts. The results show the total acid soluble phosphates to be somewhat reduced, the true inorganic phosphate to be somewhat increased, the adenosine polyphosphate fraction to be markedly reduced and the creatine phosphate fraction to be reduced.

Polarographic study of plasma protein fractions and the 'protein index.' OTTO H. MÜLLER. *Dept. of Physiology, State Univ. of New York Med. Center, Syracuse.*

In continuation of polarographic studies of the 'protein index' (MÜLLER AND DAVIS. *J. Biol. Chem.* 159: 667, 1945; *Arch. Biochem.* 15: 39, 1947; *Am. J. Med. Sci.* 220: 298, 1950) the following isolated fractions of plasma proteins have been investigated: V, IV-4, IV-1, III, II, and I. Changes in the polarographic wave height were followed as these fractions were subjected to mild alkaline digestion at room temperature for different periods of time. It was found that only fractions V, IV-1, and IV-4 produced a marked increase in wave height during the first 10–20 minutes of digestion. All fractions showed an eventual decrease in wave height with time of digestion. These observations and the fact that the fractions IV represent only a small portion of the total plasma proteins lead to the conclusion that the growth of polarographic waves observed when whole plasma is submitted to similar treatment is largely caused by fraction V (albumin). Hence this fraction must be mainly responsible for variations observed in the polarographic 'digest test'. On the other hand, analyses of sulfosalicylic acid filtrates of the various fractions following different periods of alkaline digestion show that fractions V, II, and I (albumin, γ-globulins, and fibrinogen) do *not* contribute significantly to polarographic waves similarly obtained with whole plasma. Only protein fractions III, IV-1 and IV-4 and some polypeptides present in fraction VI seem to be involved in this 'filtrate test.' These results explain why the polarographic protein index is often found to be an inverse function of the widely used albumin/globulin ratio.

Effect of insulin hypoglycemia on adrenal activity. J. SPENCER MUNROE (introduced by JAMES CAMPBELL). *Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada.*

Moderate doses of insulin caused in rats an immediate rise in the ascorbic acid content of the adrenal glands. This effect lasted about 15 minutes and was followed by a rapid and definite drop similar to that reported by Gershberg and Long. The initial level was regained in 6–8 hours. These effects were not observed when hypoglycemia was prevented by glucose. If, however, glucose was given to rats made hypoglycemic by insulin, an accelerated recovery of the ascorbic acid content of the adrenals occurred, even when the amount of sugar was not enough to cause an appreciable rise of blood sugar. In alloxan-diabetic rats a fall of blood sugar level from 650–270 mg. % within 30 minutes of insulin injection, failed to change the adrenal ascorbic acid level. The adrenal ascorbic acid was unchanged in hypophysectomised rats, and only slightly decreased in adrenal demedullated rats when insulin was given, although the blood sugar was profoundly decreased. The nitrogen content of the adrenals was usually decreased by insulin in the normal animals during these experiments (6 hours). The fall appears to be greater in the adrenal demedullated animals and was absent in the hypophysectomized group. Adrenal weight increased during these experiments, reaching a maximum within 30 minutes of injection and gradually returning to normal. These results produced by insulin hypoglycemia are consistent with the theory of the mechanism of adrenal cortical secretion described by MCDERMOTT, FRY, BROBECK AND LONG. *Yale J. Biol. & Med.* 23: 52, 1950.

Studies on the biochemistry of *Citellus*. XAVIER J. MUSACCHIA* AND CHARLES G. WILBER. *Biological Labs., St. Louis Univ., St. Louis, Mo.*

This report covers a part of the work being done on the biochemical characterization of Arctic mammals. Previous studies indicate that the blood of *Citellus barrowensis* contains relatively large amounts of lipids. Well-fed, active ground squirrels in captivity had an average blood sugar level of 153 mg/100 cc. Specimens kept at the starvation level show a mean blood reducing-sugar value of 40 mg/100 cc. During hibernation the blood sugar increases more than threefold over the starvation level. Blood nitrogen values are significantly higher for *Cittelus barrowensis* than for man or other mammals, e.g. (in mg/100 cc. serum), NPN, 65.1; urea N, 41.0; uric acid, 4.0. These values are the same in squirrels recently captured, and in those held captive for over 1 year. The amount of nitrogen excreted in the urine is not correspondingly high. Ground

squirrels kept on an ordinary laboratory diet excrete an average of 73.2 cc. of urine/24-hour period or about 100 cc/kg. body weight. The following average amounts of nitrogenous material (in mg.) are excreted daily: urea N, 89; uric acid, 1.0; creatinine, 1.3.

Interrelationship of adrenal cortex and posterior pituitary. C. SUSAN NAGAREDA (introduced by ROBERT GAUNT). *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

In certain respects the hormones of the adrenal cortex and posterior pituitary are known to have antagonistic actions but the details of the interrelationships involved are obscure. It was found in adult male rats that Pitressin in doses of 400 or 100 mU caused adrenal cortical stimulation as judged by adrenal ascorbic acid depletion. Five mU of Pitressin, a dose with definite physiological effects (antidiuresis), did not cause measurable adrenal stimulation. It was concluded, therefore, that moderate doses of Pitressin do not cause 'compensatory' adrenal activity. Another approach to the problem was based on current ideas that the rapid diuresis which follows water administration requires for its occurrence the presence of some cortical hormone and a suppression of ADH secretion. Water was given by stomach tube and adrenal ascorbic acid changes measured to determine if the induction of diuresis was associated with definite adrenal stimulation. Three ml. of water per 100 sq. cm. of body surface (= approximately 5 ml/100 gm.) caused reduction of adrenal ascorbic acid. One-half that amount of water had no effect and larger amounts had greater effects. Normal saline solution, even in large dosage (up to 9 ml/100 sq. cm. body surface in 3 doses), did not reduce adrenal ascorbic acid. The effects of non-specific manipulative factors were controlled. It was concluded that a fairly heavy water load not only inhibits posterior pituitary secretion (as per Verney and others) but also stimulates the adrenal cortex, a factor presumably contributing to the diuretic process. Neither a light water load nor saline solution caused adrenal stimulation.

Direct measurements of alveolar-arterial differences in oxygen tension in man breathing 100% oxygen. GABRIEL G. NAHAS AND EDWARD H. MORGAN (introduced by E. J. BALDES). *Mayo Fndn., Univ. of Minnesota, Rochester.*

With use of the Beckman model G2 oxygen analyzer, and the rotating platinum electrode as formerly described (MORGAN AND NAHAS. *Am. J. Physiol.* Dec., 1950), oxygen tension in whole blood and in end expiratory air was measured in 6 healthy persons breathing 100% oxygen in various positions. Vital capacity, tidal volume, expiratory reserve and blood pressure also were recorded. In the supine position, the average alveolar-arterial difference in oxygen tension was 72 mm. Hg (range 32–130), and the ventilation rate 8.2 liters per minute (range 6.3–9.6). After 2 minutes of 15 degrees backward tilt, the alveolar-arterial difference in oxygen tension averaged 63 mm. Hg (range 20–121) and the ventilation rate 7.2 liters per minute (range 5.4–10.4). After 5 minutes of 70 degrees of forward tilt, the ventilation rate rose to 9.8 liters per minute (range 7.4–11.4) and the alveolar-arterial difference averaged 87 mm. Hg (range 37–155). When the persons walked for 3–6 minutes on a horizontal treadmill at 2 mph, the ventilation rate averaged 19.3 (range 13.1–23.6) and the alveolar-arterial difference in oxygen tension was 77 mm. Hg (range 68–82). The concurrent alveolar CO_2 tension was 41 mm. Hg. The smallest oxygen gradient (26 mm. Hg, range 17–33) in relation to ventilation was obtained during hyperventilation in the supine position (average ventilation rate 18 liters per minute, alveolar CO_2 tension 24 mm. Hg).

Membrane potential changes at a single muscle end plate produced by acetylcholine. WILLIAM L. NASTUK. *Columbia Univ. College of Physicians and Surgeons, New York City.*

Small quantities of acetylcholine have been applied to a single end plate of frog muscle by means of a microneedle of pore diameter measuring approximately 1 μ at the tip. The needle was filled with acetylcholine chloride solution (110 mM/l.). For continuous recording of the resting potential the muscle cell was impaled with a fine microelectrode at a point 200 μ from the center of the end plate. As the acetylcholine-filled needle was advanced toward the end plate, a progressive depolarization of the muscle cell membrane resulted. When the depolarization amounted to about 20 mv. the position of the needle was fixed. During the succeeding 10–30 seconds the membrane potential usually remained within 2–3 mv. of this value although in a few cases the membrane showed a marked repolarization (8–10 mv.). When the needle was removed, the cell membrane repolarized to a new level in approximately 12 seconds and remained at this level for the remainder of the time of recording (1–4 minutes). The resting potential at this level averaged 82 mv. as compared with the control value of 91 mv. In a few experiments the end plate was depolarized to the extent of 36 mv. at which point repetitive action potentials were produced. Repetitive firing could be obtained in several successive trials by alternately moving the needle toward and away from the end plate. Attempts to depolarize the muscle cell membrane at a point distant from the end plate gave little or no result.

Inhibition of dimethyl-p-aminoazobenzene induced hepatomas. DOROTHY NELSON*, PAUL SZANTO* AND A. C. IVY. *Dept. of Clin. Science, Univ. of Illinois College of Medicine, Chicago and Hektoen Inst., Chicago.*

Nutritional inhibitors of the hepatomas induced by feeding dimethyl-p-aminoazobenzene (DAB) are thought to influence the metabolism of the liver rather than that of the dye. Therefore we were interested in the tumor inhibiting effect of two preparations which stimulate hepatic regeneration. These substances are nucleic acid and the VioBin liver residue after the extraction of both fat and water soluble vitamins. We compared these with a liver extract containing the vitamin B complex and known to afford protection against DAB. Four groups of 20 adult rats received the following diets: the control diet contained casein (12), dextrin (42), sucrose (25), fortified corn oil (12), yeast (5), salt (4), DAB (0.06). The experimental diets substituted liver residue (28), extract (4), or nucleic acid (0.65) for equivalent dextrin. After 28 weeks half of the control animals had palpable tumors or had died with tumors. The experiment was continued for 4 additional weeks without the dye in the diets. The surviving animals were sacrificed and the pertinent tissues examined and preserved. As judged by the number and size of the tumors and the condition of the non-tumorous portion of the livers, the liver residue and the liver extract gave marked protection. The residue was somewhat better than the extract but the difference was not statistically significant. The number of tumors in the animals receiving nucleic acid was only 18% less than the controls but they were very much smaller.

Comparison of erythropoietic response in normal and adrenalectomized rats following hemorrhage. ROBERT NELSON (introduced by THOMAS H. ALLEN). *Dept. of Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

The ability of bilaterally adrenalectomized rats to produce red blood cells was determined by using a hemorrhagic stress. Two cubic centimeters of blood per 100 gm. of body weight were removed by heart puncture from rats adrenalectomized approximately 19 days previously. Adrenalectomized animals were given 0.9% NaCl in their drinking water. The hematocrit and reticulocyte percentages were determined daily for 10–14 days following hemorrhage. Measures were taken to prevent undue blood loss as a result of sampling. Although in the adrenalectomized rats the average pre-hemorrhage hematocrit value was slightly lower than in the control animals, both operated and control animals restored their respective pre-hemorrhage hematocrit levels within 10 days. Also the average reticulocyte responses were similar in pattern and magnitude. Adrenalectomy was confirmed by gross autopsy and histologic examination of suspected tissue. Under the conditions of this study there was no evidence that adrenalectomy impairs erythropoiesis following hemorrhage.

Estrogen secretion by the testes. WARREN O. NELSON. *College of Medicine, Univ. of Iowa, Iowa City.*

It has been contended by various workers that the site of estrogen production in the testis is the Sertoli cell (Huggins and Moulder, 1945; Teilum, 1950 and others). On the other hand recent studies by Elftman (1950) emphasized that Sertoli cell activity is related directly to sperm maturation and is probably nutritional. Material and observations have been assembled on 5 individuals, ostensibly female, which bear on the problem of the site of estrogen production in the testis. Each of these individuals showed feminine breast development and normal feminine body habitus. Three of them were married. Sexual hair and external genitalia were variable. Their gonads, present in the inguinal canal or abdomen, showed the gross and microscopic characteristics of testes. In no instance was spermatogenesis in progress; in three the Sertoli cells were in an essentially undifferentiated condition while in two, many of the Sertoli cells appeared to be reasonably normal. All showed numerous fully differentiated Leydig cells. Removal of the gonads was followed by a drop in the level of estrogen excretion and by estrogen withdrawal symptoms (breast, vagina etc.) Various histologic and histochemical studies suggest that if estrogen was being produced by these gonads the site of secretion is more likely to have been the Leydig cells than the Sertoli cells. This seems particularly probable in the three instances in which the Sertoli cells were undifferentiated. It is suggested that more evidence must be offered before the Sertoli cell can be considered to be a site of hormone production in the human testis.

Direct inactivation of gonadotrophin thyrotrophin and prolactin by extracts of lithospermum ruderale. R. L. NOBLE, E. R. PLUNKETT* AND R. C. GRAHAM.* *Dept. of Med. Research, Univ. of Western Ontario, London, Ontario, Canada.*

In addition to the marked inhibition of separately injected gonadotrophins, lyophilized water extracts of the plant lithospermum ruderale in one-fiftieth the amount cause almost complete inhibition when mixed *in vitro* and incubated for 2 hours at 37°C. prior to injection. Pregnant mare's serum gonadotrophin shows the most marked susceptibility to *in vitro* inactivation,

and when assayed on hypophysectomized immature female rats, shows no sign of activity remaining. The gonadotrophin of crude rat pituitary and pregnancy urine are also inhibited but to a lesser degree. Daily doses of a prolactin extract, capable of more than doubling the crop gland weight of pigeons were markedly inhibited by mixing and incubating with 0.8 mg. of lithosperm. Similar *in vitro* inhibition of thyrotrophin was obtained when assayed on the thyroid response of the young guinea pig. Other studies indicated that posterior pituitary extracts and insulin were unaffected by lithosperm, while adrenaline was inactivated.

Retinal potentials during metabolic impairment. WERNER K. NOELL. *USAF School of Aviation Medicine, Randolph Field, Tex.*

Specific effects of iodoacetate, azide and anoxia on mammalian retina enable demonstration that, *a*) the chain of reactions which supports the electroretinogram differs from that which maintains the resting potential of the eye; *b*) the c-wave of the electroretinogram (or potentials corresponding in time) and the resting potential are closely correlated with respect to size and polarity; *c*) the spontaneous optic nerve discharges are partly maintained by processes in the outer layers of the retina; and *d*) the transient rise of the resting potential induced by azide (*Federation Proc.* 9: 95, 1950) is associated with changes in spontaneous discharge rate depending, within limits, on properties of the fiber as revealed by the response to illumination. 'On'-elements which show lasting discharges during illumination tend to respond with a small or moderate increase of their discharge rate during the rise of the resting potential and with a depression of discharge rate while the resting potential turns to decline. 'Off'-elements respond with an increase of discharge rate predominantly after the peak of the resting potential rise; but only a few of these elements exhibit a depression during the rising phase. With high doses of azide every fiber discharges more frequently during the declining phase. Illumination suppresses the discharge response during this phase and releases it after 'off' whereby the effectiveness of suppression depends on the normal responsiveness of the fiber to photic stimulation. Pathways normally inhibited by illumination may transiently become excitatory ones during a late phase of marked depression following a strong discharge response.

Age differences in respiratory ventilation and oxygen consumption following standardized exercise. ARTHUR H. NORRIS,* MARVIN J. YIENGST* AND NATHAN W. SHOCK. *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and Section on Gerontology, Baltimore City Hosps., Baltimore, Md.*

Excess ventilation volume and oxygen consumption following exercise were measured in 5 subjects 25–29 years of age, 5 aged 62–77, and 4 aged 80–85. Subject selection was based on ability to perform the work required and absence of clinical evidence of disabling cardiovascular disease. Arm exercise was performed on a calibrated ergometer with recumbent subject. Four amounts of work (135–600 kg. m.) at a constant rate of 450 kg. m/minute and a constant amount of work (500 kg. m.) at four different rates (135–585 kg. m/minute) were performed by each subject on different days with continuous collection of expired air for 40 minutes after exercise. The aged subjects were unable to perform at work loads over 500 kg. m. and rates greater than 450 kg. m/minute. With increasing total work loads (constant rate) each age group showed greater increase in excess ventilation than the next younger group and the old group showed greater increase in excess oxygen consumed than the two younger groups in which excess O_2 was the same. Each group showed greater excess ventilation than the next younger group at all rates of work. With increasing work rates excess ventilation increased in the young and middle aged but was unchanged in the aged for the rates of work they could perform (450 kg. m/minute). The excess oxygen consumed diminished with increasing work rates. The reduction was greatest in the oldest group.

Effect of anoxia on intestine of albino and hooded rats. DAVID W. NORTHUP, EDWARD J. VAN LIERE AND J. CLIFFORD STICKNEY. *Dept. of Physiology, West Virginia Univ. School of Medicine, Morgantown.*

Previous work (in press) has shown that a series of painful stimuli (electric shocks) decreased propulsive motility of the small intestine in the albino rat but not in the hooded rat. It has also been previously shown (VAN LIERE, E. J., W. V. CRABTREE, D. W. NORTHUP AND J. C. STICKNEY. *Proc. Soc. Exper. Biol. & Med.* 67: 331, 1948) that anoxia inhibits motility in the albino. In the present study hooded and albino rats were subjected to anoxia (simulated altitude 24,000 feet) to determine any difference in their response to this type of stress. Two hooded and two albino rats were given 2 cc. of an acacia-charcoal mixture by stomach; 5 minutes later one hooded and one albino were placed in the decompression chamber while the controls remained outside; after 35 minutes of anoxia for the experimental rats the animals were all decapitated and the length of small intestine traversed by charcoal was measured. There were 10 rats in each category. Average distance traversed, in percentage of total length of

small intestine: albino controls, 71%; albino anoxic, 49%; hooded controls, 73%; hooded anoxic, 45%. There is no significant difference in the effect of anoxia on the propulsive motility of the small intestine in hooded and albino rats.

Effect of veratrone on blood pressure and renal hemodynamics of normal and hypertensive dogs. WILLIAM C. NUNGESSER,* AND EDWIN P. HIATT. *Depts. of Physiology and Pharmacology, Univ. of North Carolina School of Medicine, Chapel Hill.*

A study is being made of the effects of veratrone (Parke, Davis & Co.) a non-alcoholic solution of veratrum viride alkaloids, on the circulation of normal and hypertensive dogs. The animals are unanesthetized trained female mongrels and the hypertensive dogs are of two types, neurogenic and renogenic. The drug is administered subcutaneously in doses of 0.025 mg. of alkaloid per kg. of body weight. Blood pressures are recorded from femoral artery punctures. Glomerular filtration rate and effective renal plasma flow are measured by clearance methods using exogenous creatinine and p-aminohippurate respectively. In all 3 types of dogs there is a significant fall in blood pressure beginning in less than one hour. Bradycardia is usual but not invariable. The depressor effect is not due entirely to the cardiac slowing but reflects a vasodilatation as well. In the normal dogs and in the neurogenic hypertensives there is an increase in renal plasma flow and glomerular filtration rate, the consistency and magnitude of this effect being greater in the hypertensives. There is no definite trend in the changes in filtration fraction in the normal animals, but in the neurogenic hypertensives there is usually a slight decrease. In the renal hypertensives the blood pressure fall and the bradycardia are more marked. The renal plasma flow and the glomerular filtration rate decrease markedly in sharp contrast to the normal and neurogenic hypertensives and the filtration fraction usually decreases.

Photoöxidation of crystalline estrogens in the presence of flavins. JOSEPH F. NYC,* HARRY B. FRIEDGOOD, JOSEPHINE B. GARST* AND DOROTHY M. MARON.* *Depts. of Medicine and Physiological Chemistry, Univ. of California, Los Angeles; California Inst. for Cancer Research, Los Angeles.*

Spectrophotometric data show that estrone, estradiol and estriol in aqueous alcoholic solution undergo marked changes in the ultraviolet region when these steroids are exposed to light in the presence of riboflavin or lumichrome. Our experiments also demonstrate that photoöxidation of estrone in the presence of riboflavin results in a large loss of its biological activity as determined by a modification of the Doisy vaginal smear method. Other observations in this series of experiments have revealed that manganous chloride in a molar concentration of 10^{-2} inhibits the photodestruction of these naturally occurring estrogens. The available evidence derived from the foregoing studies with crystalline estrogens suggests that current methods for the quantitative extraction from urine and blood of estrogens, and possibly other steroid substances, may be subject to errors which have been overlooked heretofore.

Some pharmacological effects of N 3-4-5 trimethoxyphenylethyl mescaline. MAURICE C. OLIVIER (introduced by C. H. THIENES). *Dept. of Pharmacology, Univ. of Southern California Med. School, Los Angeles.*

On the urethanized rat, bis (3-4-5 trimethoxyphenylethyl) amine hydrochloride, injected intravenously in doses of 3.3 mg/kg., was found to reverse or inhibit the pressor effect of small doses (1–5 μg/kg.) of epinephrine injected intravenously. It also inhibits the pressor effects of l-arterenol. It was noted that 4 to 8 times the amount of l-arterenol had to be administered to obtain the same blood pressure increase as with l-epinephrine. On the isolated small intestine of the rat, the compound was found to have an inhibitory effect on the relaxing action of epinephrine when administered prior to the latter substance. It was also found to prevent the characteristic rapid contraction of the isolated rat ventricle strip when administered before doses of epinephrine which otherwise would initiate this type of contraction. The LD_{50} of the compound in mice injected intravenously was found to be 120 mg/kg.

Oxidations mediated by normotensive and hypertensive tissues. NORMAN S. OLSEN. *Hypertension Div., Washington Univ. School of Medicine, St. Louis, Mo., and Research Lab., Thayer VA Hosp., Nashville, Tenn.*

Previous studies on the metabolism of renal and other tissues of hypertensive animals have given contradictory results. In the present investigation rats were made hypertensive by constriction of the left renal artery. The oxygen uptake of homogenates of both kidneys of hypertensive animals did not differ from normotensive values. However, the oxygen uptake of left kidney (constricted) homogenates was significantly lower than normal, while homogenates of right kidneys of hypertensive animals were somewhat above the normal. This difference can be partially explained on a chemical and histological basis. Added amino acids and their corresponding amines as substrates were oxidized to a lesser extent by homogenates of hypertensive kidneys than corresponding homogenates from normotensive kidneys.

Using liver preparations no differences were noted in any of the parameters for the two groups of animals. These results will be discussed in relation to various theories of renal hypertension.

Effect of total sympathectomy on hemoglobin production of dogs. PETER D. ORAHOVATS* AND WALTER S. ROOT. *Dept. of Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

Normal and sympathectomized dogs fed a standard diet to which iron was added, were maintained in a constant anemic state by frequent bleedings (Whipple's method) for periods of 3–4 months. The hemoglobin concentration in both groups of animals was reduced from 13.5–18.4 to the anemic level 6.8–8.1 gm/100 cc. of blood. Hemoglobin, hematocrit and plasma proteins were measured every other day. Hemoglobin was estimated spectrophotometrically as acid hematin being read against a standard, prepared from normal dog blood of known oxygen capacity (Van Slyke). Blood volumes and body weight were determined regularly. Sympathectomies were done as described by Cannon. Four to 6 weeks after the final operation the animals had regained their preoperative hemoglobin and hematocrit values. These dogs were bled as described above. Then, the completeness of sympathectomy was tested by occluding the exposed carotid arteries. The absence of an increase in blood pressure was taken as evidence that sympathetic regeneration had not occurred. In calculating the hemoglobin production, the results of the first month of bleeding were not included. The 5 anemic, non-sympathectomized dogs show a mean hemoglobin production of 0.59 gm/kg. body weight/day compared with a mean value of 0.55 gm/kg. body weight/day produced by the 5 sympathectomized animals.

Effect of cortisone and DCA on Na^{24} transport in normal dogs. R. R. OVERMAN, A. K. DAVIS* AND ANNE C. BASS.* *Div. of Physiology, Univ. of Tennessee College of Medicine, Memphis.*

Measurements of the following variables were made in *1*) 6 normal dogs, *2*) 4 normal dogs given 5.0 mg/kg/day of cortisone for 7 days and *3*) 5 normal dogs given 0.5 mg/kg/day of DCA for 7 days: total Na, K, Cl and H_2O concentrations in plasma, packed erythrocytes, liver, muscle, heart, gut, spleen, lung, skin, brain and (Na only) bone; Na^{24} concentrations 1 minute following i.v. injection of 1 millicurie Na^{24} and at 6-minute intervals thereafter for 30 minutes in plasma and packed erythrocytes; Na^{24} concentrations in the tissues listed above 30 minutes after Na^{24} injection; plasma volume, SCN volume and hematocrit. Average ionic concentrations in these groups in mEq/l. were as follows: cortisone series—plasma Na 142, K 4.5, Cl 109; erythrocyte Na 113, K 5.8, Cl 70; DCA series—plasma Na 153, K 3.5, Cl 113; erythrocyte Na 125, K 5.2, Cl 62. The erythrocyte Na turnover rates were increased 42% in the cortisone series and 45% in the DCA group. Relative specific activity figures for each tissue listed above indicated that the tissue Na turnover rate per unit of Na is uniformly reduced in normal dogs whether they receive cortisone or DCA. Quantitatively, however, large differences were found between the 2 series. The average percentage reduction in relative specific activity of tissues in the DCA series was 21 (range 11–44) while that of the cortisone group was but 10 (range 4–16).

Effect of cortisone and DCA on Na^{24} transport in adrenalectomized dogs. R. R. OVERMAN, A. K. DAVIS* AND ANNE C. BASS.* *Div. of Physiology, Univ. of Tennessee College of Medicine, Memphis.*

Measurements of the variables listed in the preceding abstract were made in *1*) 5 adrenalectomized dogs given 5.0 mg/kg/day of cortisone for 7 days and *2*) 5 adrenalectomized dogs given 0.5 mg/kg/day of DCA for 7 days. Average ionic concentrations in these groups in mEq/l. were as follows: cortisone series—plasma Na 138, K 5.7, Cl 103; erythrocyte Na 111, K 5.9, Cl 62; DCA series—plasma Na 144, K 3.2, Cl 114; erythrocyte Na 114, K 4.7, Cl 74. The erythrocyte Na turnover rate was reduced 15% in the DCA series and increased 12% in the cortisone group. Among the variables measured there were other instances of essentially opposite actions of the 2 hormones and many indications that the various tissues are affected differently—both quantitatively and qualitatively by DCA and cortisone. Thus, while DCA produced increments in tissue Na in liver, heart and lung, cortisone produced reductions. In spleen, the opposite occurred. And although DCA increased the Cl space of liver and reduced it in muscle, cortisone reduced the Cl space of liver and increased that of muscle. Relative specific activity figures indicate that with the exception of spleen and bone, an average increase of 11% in tissue Na turnover rate per unit of Na occurred (range 6–19) in the DCA series. Cortisone produced without exception a reduction in tissue relative specific activity (average reduction 18%, range 5–46).

Fluid balance studies in gut, muscle and skin of rats after total body x-radiation. E. E. PAINTER AND M. A. COOLEY.* *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Analyses of gut, muscle and skin of 220-gm. Sprague-Dawley male rats before and after a single dose of total body x-radiation (LD_{50}) indicate that these tissues show no similarity in degree or direction of their electrolyte changes (based

on plasma and tissue Na, K, Cl, H_2O). In gut after radiation there is a rise in extracellular space and a fall in intracellular space associated with a fall in intracellular K. In muscle, not as severely damaged as gut after radiation, there is a decrease in extracellular space and a slight rise in intracellular space with no significant change in intracellular K. In skin there is a decrease in intracellular K, but no change in the fluid compartments. To what extent cellular breakdown per se rather than alteration in endocrine function accounts for these findings is being investigated. All data were based upon 10 animals or more and statistically handled.

Conventional methods compared with an absolute method for pressure transducer response measurement. JOHN PARNELL, E. L. BECKMAN AND THOMAS PEIRCE (introduced by J. R. POPPEN). *Moore School of Electrical Engineering and Dept. of Physiology, School of Medicine, Univ. of Pennsylvania, Philadelphia, and Aviation Med. Acceleration Lab. Naval Air Development Center, Johnsville, Pa.*

The two common methods of pressure transducer evaluation are: *1*) the amplitude-frequency response to a pressure, sine wave, and *2*) the response to a pressure step function. In the first method, the response of the test transducer to a pressure sine wave is measured. The sine wave is obtained from a reproducer and is monitored by a transducer of assumed calibration. In the second method, the response of the system to a step function is measured. The amplitude-frequency characteristic may be calculated from the step function data if it is assumed that the system has but one degree of freedom. This assumption is not always valid. A method for absolute calibration of pressure transducer systems based on the application of the reciprocity theorem was developed by scientists working with underwater sound radiation and detection during the last war. The Navy Underwater Sound Reference Laboratory is equipped for the absolute calibration of underwater sound apparatus. Absolute calibration of several physiological pressure transducer systems was obtained using these facilities. Besides the absolute calibration, each system was calibrated by the two conventional methods insofar as was possible. The amplitude-frequency characteristics obtained using conventional methods were in general agreement with those obtained using the absolute method.

In vitro inactivation of cortisone by tissues of normal and stressed adrenalectomized rats. KARL E. PASCHKIS, A. CANTAROW AND D. BOYLE.* *Jefferson Med. College, Philadelphia, Pa.*

The fact that stress increases the requirement for adrenocortical hormone is generally recognized. How this extra amount of cortical hormone is used is, however, unknown. It is frequently stated, although without adequate evidence, that the rate of 'utilization' of cortical hormones is increased under conditions of stress. Data are presented in this paper on the 'inactivation' *in vitro* of compound E (cortisone) by tissue slices and tissue homogenates from normal and from adrenalectomized stressed rats. Tissues were incubated with cortisone and the latter was extracted and assayed by the mouse-liver glycogen method of E. Venning. Boiled tissue incubated with cortisone served as a control. In contradistinction to the preponderant role of the liver in inactivating estrogens and androgens, other tissues, such as striated muscle (diaphragm) and brain, appear to be as effective as liver in inactivating cortisone. Under the conditions of our experiments 15–35% of the activity of cortisone is lost. The activity of liver homogenates does not differ significantly from that of slices; addition of DPN does not increase the activity of liver homogenates. The activity of tissues from stressed adrenalectomized animals, appears to be of the same order of magnitude as that of normal animals.

Studies on progesterone metabolism in cases of liver disease. K. E. PASCHKIS, A. CANTAROW AND W. P. HAVENS, JR.* *Jefferson Med. College, Philadelphia, Pa.*

Six patients with severe hepatic disease received progesterone, 100 mg. daily, for 3–4 weeks by intramuscular route. Urinary pregnanediol was determined by the method of Sommerville and Marrian at about weekly intervals. The excretion appeared to be high, in one instance 52 mg. in 24 hours. The '17-ketosteroid' excretion was determined before and during treatment, using the method of Callow and Callow. In 3 of the 6 cases, Zimmerman positive steroids were increased, with appreciable increase in the free moiety. Two patients, one with cirrhosis and one with rheumatoid spondylarthritis, received 100 mg. progesterone daily intramuscularly on 3 successive days. Pregnanediol excretion was determined before, and for 9 days after injection. The total recovery was 46% of the injected progesterone in the cirrhotic and 45% in the arthritic. Total excretion of pregnanediol is even higher, since excretion levels were still found high on the 9th day. The high recovery of pregnanediol in the arthritic confirms similar findings by Marrian and Sommerville; we observed a similarly high excretion in hepatic disease. This suggests that the metabolism of pregnanediol is impaired in the presence of hepatic disease. Formation of pregnanediol from progesterone is perhaps either unimpaired, or a

small impairment may be masked by the impaired degradation and consequent increased excretion of pregnanediol. The increase in Zimmerman positive steroids suggests the possibility that 20-ketosteroids such as Pregnane 3 (a)-ol-20-one are formed in detectable amounts. The increase of the free moiety is similar to the increase of free 17-ketosteroids found by us in cases of hepatic disease after administration of testosterone.

Studies on differential circulatory effects of carbon dioxide. JOHN L. PATTERSON, JR., EDWARD J. WAITS AND MARY V. MCPHAUL (introduced by JOHN HALDI). *Emory Univ. School of Medicine, Atlanta, Ga.*

The vascular effects of high concentrations of carbon dioxide are believed to be due to chemoreceptor and vasomotor center stimulation together with a more direct action upon blood vessels themselves. In the present studies on dogs, the attempt was made to separate these actions by the use of tetraethylammonium chloride (TEAC) in dosage sufficient to cause complete ganglionic blockade. In 24 experiments, the effects of inhalation of the mixture: (5% CO_2, 21% O_2, 74% N_2) as compared with room-air control observations, on arterial pressure (electromanometer), blood flow in the hind limb (air plethysmograph) and cardiac output (T-1824 dye) were determined. In the chloralosed animals not given TEAC, carbon dioxide regularly produced a rise in mean arterial pressure of from 3–25 mm. Hg, and a fall in limb flow varying from 6–50%. Cardiac output either fell slightly or was unchanged. In the animals given TEAC, carbon dioxide produced some opposite effects (determinations during stable TEAC effect considered as baseline): a fall in arterial pressure of from 4–23 mm. Hg; a rise in limb flow of 8–84%. Cardiac output usually fell moderately. These studies suggest that, in a sense, the 'direct' action of CO_2 and that mediated by nervous mechanisms are in competition. The fall in cardiac output with an associated rise in limb flow suggests the 'direct' dilatory effect of CO_2 on limb vessels must not be representative of the entire vascular system.

Relation of motor to secretory function in stomach of fish. T. L. PATTERSON. *Hopkins Marine Station, Stanford Univ., Pacific Grove, Calif., and Wayne Univ. College of Medicine, Detroit, Mich.*

This report is one of a long series of phylogenetic studies on the comparative physiology of the gastric motor mechanism of vertebrate and invertebrate animals. A sedentary, marine fish, the bullhead or cabezone (*Scorpaenichthys marmoratus*), one of the most common of the shallow-water sculpins was used. Following anaesthetization, each fish was stomostomized and placed separately in an aquarium containing fresh, aerated, running sea water. Gastric motility studies (balloon method) previously reported have shown that the digestive and hunger motility of this fish is of a continuous, rhythmical type, the contractions being somewhat stronger during digestion than during hunger peristalsis. A definite periodicity does not exist although there is a modified type. In the present investigation, both the motor and secretory functions of the stomach were synchronously and graphically recorded. The usual balloon method was employed for registering the gastric motility and the gastric secretion was conveyed through a rubber tube with perforations at the stomachic end to a drop recorder and receptacle for collection and testing of the gastric juice. The existing experimental conditions here, because of the character of the dual recording, must of necessity lead to a restricted space for movements of the cabezone but this does not seem to effect the well being of the fish which is probably due to its sedentary nature. With this new technique, gastric motility and gastric secretion may be synchronously recorded, the secretion running parallel to the motility periods.

Acute hemorrhagic pulmonary edema from hypothalamic lesions in rats. HARRY D. PATTON AND J. E. GAMBLE.* *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Bilateral electrolytic lesions in the rostral hypothalamus produced fatal hemorrhagic pulmonary edema in 18 rats and marked pulmonary pathology in 15 other rats sacrificed 24 hours postoperatively. The former usually died within 8 hours after recovery from anesthesia (Evipal); the final crisis was often explosive with copious discharge of pink-tinged, frothy fluid from the respiratory passages (1.5–2.4 cc.). (A proteinaceous pleural effusion was found in 7 animals.) The lungs were dark red and swollen; the cut surfaces exuded fluid. In most fatal cases all lobes were involved; in sacrificed rats the discoloration was often distributed irregularly. Microscopic examination showed distention of the alveoli and perivascular spaces with erythrocytes and fluid. Dry and wet lung weights and lung weight/body weight ratios were significantly greater than mean values for 72 control animals with non-edemagenic hypothalamic lesions. The pulmonary changes were not secondary to hyperthermia; some rats with normal or subnormal body temperatures developed edema. Thirteen rats with postoperative hyperthermia and 10 rats with diathermy-induced hyperthermia showed only mild discoloration and congestion of the lungs. The brain lesions in most of the edematous animals were in the preoptic

region overlying the rostral half of the chiasm and centering a millimeter from the midline. In 4 edematous animals with asymetrical caudal lesions, one midline needle tract damaged the periventricular system bilaterally. This suggests that the executant fibers course caudally near the ventricle. Study of brains from animals with nonedemagenic hypothalamic lesions demonstrated that neither bilateral damage bordering the preoptic region nor unilateral preoptic damage produced pulmonary edema.

Relationship of pituitary and adrenal cortex to ovarian hyperemia reaction in the immature rat. R. W. PAYNE (introduced by R. O. GREEP). *Ziskind Research Lab. of New England Center Hosp. and Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Adrenalectomy prevented the rapid hyperemia response of the immature rat ovary to both human chorionic gonadotropin and a gonadotropic anterior pituitary extract. Cortisone acetate restored this response to both substances. Hypophysectomy prevented the ovarian hyperemia response to chorionic gonadotropin in doses as high as 500 times the minimal effective dose for intact animals but did not abolish ovarian hyperemia in response to the anterior pituitary preparation. Cortisone acetate and corticotropin restored the effect in hypophysectomized animals treated with chorionic gonadotropin. Cortisone acetate alone in doses of 1.25 mg. did not produce hyperemia of the ovary in intact, adrenalectomized, or hypophysectomized animals. A partially purified corticotropin in doses as high as 10 mg. did not produce ovarian hyperemia in intact animals. Cortisone acetate in doses as small as 10 μg. restored the ovarian hyperemia potentiality in adrenalectomized animals treated with both gonadotropins used and in hypophysectomized animals treated with chorionic gonadotropin. Progesterone in oil and desoxycorticosterone in oil in much larger doses produced this effect. Testosterone proprionate in oil, stilbestrol in oil, and pregnenolone as an aqueous suspension were ineffective in doses as high as 2.5 mg. No synergism between cortisone acetate and chorionic gonadotropin was shown in intact animals as subhyperemic doses of human chorionic gonadotropin were not potentiated by large doses of cortisone acetate. The evidence presented indicates that the adrenal cortex conditions the ovary for the development of hyperemia in response to gonadotropins.

Reversible inactivation of crystalline urease, brain succinoxidase and rat brain respiration by 2,4-dinitrophenol. C. N. PEISS (introduced by F. E. FRANKE). *Dept. of Biology, Johns Hopkins Univ., Baltimore, Md.*

Crystalline urease, dissolved in 2×10^{-1}M dinitrophenol (DNP) to give a solution containing 16,000 gamma urease/ml., shows no demonstrable urease activity when measured manometrically in acetate buffer at pH 5.0. Control solutions, prepared in water and suitably diluted, yield Q_{CO_2} values of 2400–2900. Dilution of the totally inactivated urease with water, thereby reducing urease concentration to a measurable level in the respirometer flask, and reducing DNP concentration to 2×10^{-4}M, results in reactivation of urease, with Q_{CO_2} values of 2200–2800. Similar results have been obtained using various concentrations of urease, and varying degrees of inactivation by modifying DNP concentration. Whole rat brain was soaked in ice-cold water containing 2×10^{-2}M DNP for a period of 10 minutes. The brain was then homogenized in the same solution; the succinoxidase Q_{O_2} was 16. The other half of the brain was washed several times and then homogenized in cold water. The resultant Q_{O_2} for succinoxidase was 37. Control Q_{O_2}'s ranged from 39–41. Thus, DNP inhibition of brain succinoxidase is reversed 90–95% by washing. Rat cerebral cortex slices, soaked 20 minutes in Ringer solution containing 2×10^{-2}M DNP, and suspended in this solution in respirometer flasks, show no oxygen uptake. Slices treated identically, and then washed repeatedly with cold DNP-free Ringer solution, gave a Q_{O_2} of 5–6. Control slices have a Q_{O_2} of 10–11. This indicates about 50% reversal of DNP inhibition of rat cerebral cortex respiration by washing.

Regional rates of cutaneous insensible perspiration. C. N. PEISS,* A. B. HERTZMAN, W. C. RANDALL AND H. E. EDERSTROM. *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Regional evaporative rates from shaved skin areas were measured by the desiccating capsule technique on nude subjects exposed to an operative temperature of 27°C. ('still' air). Absence of detectable sweating in the observed areas was verified by the iodine-starch-paper technique; onset of sweating was indicated simultaneously by the two methods. Low rates (.007 mg/cm²/min.) of the movement of water of diffusion are exhibited uniformly over the skin of the arm, leg and trunk; high rates are shown on the palm (.07–.15), sole (.05) and face (.04). Palmar evaporation without indication of sweat gland activity may take place at rates comparable to free thermoregulatory sweating in such areas as the calf and thigh. Calculation of total cutaneous insensible perspiration from the weighted (DuBois weighting) regional rates yields values (6.8 kg. cal/m²/hr.) closely approximating those estimated from losses of body weight (BURCH AND WINSOR). Values higher

than this are accompanied by demonstrable sweating. The rates of water of diffusion correlate with local blood supply. The application of diffusion theory to the calculations of surface vapor pressures in the absence of liquid water and the performance of the desiccating capsule technique will be discussed. (Aided by contract with the Air Materiel Command, Army Air Forces).

Effect of *g* upon absorption of P^{32} in the monkey. V. A. PERTZOFF (introduced by S. W. BRITTON). *Physiological Lab., Univ. of Virginia, Charlottesville.*

The moneky, *Macacus rhesus*, was injected i.p. with 10 μc. of P^{32}. The rate of absorption and appearance in the blood stream was measured by a G.M. counter fixed over the anterior surface of the wrist, the animal being supine with both forelimbs extended above the head. Comparative runs were made with the animal at rest and under the influences (20 minutes) of a gravitational field (centrifuge). It was found that 1 *g* has no influence upon this rate. Two *g* lowered the rate of absorption by a factor of about 4. Epinephrine (0.2 cc. 1/1000 i.p.) lowered the rate of absorption by a factor of about 2. Injection of histamine had no effect.

Conditioning of human heart rate to graded degrees of muscular tension. JOHN E. PETERS* AND W. HORSLEY GANTT. *Pavlovian Lab., Johns Hopkins Univ., Baltimore, Md.*

Previous experiments from this laboratory (*Federation Proc.* 7, 1948) showed cardiac increase during muscular activity in the human proportional to degree of muscular tension. In the present experiments, maximal exertion in 11 subjects was determined by a hand dynamometer. Three different colored lights indicated to the subjects whether to exert maximum, one-half maximum, one-sixth maximum exertion as shown on the dynamometer after 10 seconds' duration of the appropriate light. After some repetitions of light plus exertion the subjects developed conditioned heart rates; 8 showed an increase (see table), 3 a decrease during the signal for exertion.

	MAXIMUM TENSION			½ MAXIMUM			⅙ MAXIMUM		
	Control	CR	UR	Control	CR	UR	Control	CR	UR
Av. heart rate	82.0	87.5	102.1	78.5	82.3	94.	79.3	82.5	84.8

The subjects characterized by deceleration had only a slight decrease and about the same to all 3 tensions—2–4/minute. Conclusion: a cardiac conditional reflex can be established in humans to exertion as readily as in dogs to pain or food; it is in proportion to the exertion that the conditional signal represents. Human subjects as well as dogs fall into two types: a majority show accelerated cardiac conditional reflexes; a few show decelerated cardiac conditional reflexes.

Participation of the veins in active regulation of circulation. LYSLE H. PETERSON. *Dept. of Physiology, Med. School, Univ. of Pennsylvania, Philadelphia.*

This investigation was designed in an attempt to obtain quantitative information regarding the extent to which the vein walls themselves contribute actively to the regulation of the circulation. The approach has been to induce within the vein a pressure wave by injecting a pulse of a known volume of saline in a known time period. The volume pulse is introduced into the vein through a catheter and the resulting pressure pulse wave shape, its transmission and distortion are recorded from two additional catheters lying in various related segments of the vessel. The volume-time relationships of the volume pulse are of importance in forming an analyzable pressure pulse. In this procedure the volume-pressure ratio, pulse wave velocity, venous pressure and venous pulse is recorded in various segments. From these data one should obtain information indicative of changes in distensibility (tone), volume and flow relationships in the vein. To date this technique has been limited to the great veins of anesthetized dogs, however, it appears practicable for use in man. The pulse wave velocity varies between 0.5 and 1.0 meters per second. In addition to the respiratory variations in the characteristics mentioned above, there appear to be low frequency undulations. Hemorrhage studies are preliminary and the correlation of venous changes with other events have not been completed, however, there appear to be marked alterations in the characteristics of the venous walls. Following death, rapid and marked changes occur indicating an increased distensibility.

Stimulation of acid secretion by infusion of acetylcholine into arteries of the fundus of the stomach. LEO PEVSNER (introduced by GLADYS BUCHER). *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.*

Several investigators have reported that infusion of acetylcholine (ACh) solution into arteries supplying the greater curvature of the stomach evokes an alkaline mucoid secretion. In the present study these experiments were repeated in dogs and a dose range was found which consistently evoked a watery secretion containing acid. The technique included complete separation of the pyloric gastrin secreting portion of the stomach from the fundus with closure of the former and cannulation of the latter. A branch of the splenic artery was then isolated. The animal was allowed to recover and a day or two later the isolated artery was drawn out through the incision, cannulated and infused with ACh solutions by means of a motor driven pump at a rate of 120 cc/hour. Doses ranging from

0.006 through 0.6 mg/hour produced acid secretion whereas higher doses resulted in inhibition and return to mucoid consistency. Maximal acid secretion was obtained at 0.06 mg. of ACh/hour. The maximal rate of secretion ranged from 22–86 cc/hour and free acid concentration from 13–120 mEq/liter. Returning from higher inhibiting doses to the stimulating dose range again evoked acid secretion. Our work differed from that of previous investigators in that first, we used unanesthetized animals and second, we used considerably lower doses of ACh. Our results support the view that acetylcholine is the neurohumoral mediator for vagal stimulation of gastric acid secretion.

Gross composition of fat-free mammalian body. GROVER C. PITTS (introduced by S. W. BRITTON). *Physiological Lab., Univ. of Virginia, Charlottesville.*

Several lines of indirect evidence indicate a constancy in gross composition of the fat-free body. The present work is a test of this constancy in the normal mammal. The mass of the fat-free body is estimated by two independent methods *1*) determination of body specific gravity by underwater weighing and *2*) determination of total body water by the method of antipyrine dilution. The fat-free body mass is related to the mass of the following components: bone, skin, muscle and blood. Bone, skin and muscle are determined by direct gravimetric methods on guinea pigs, and blood is determined on man by dilution of the dye T-1824. Results to date appear to support the predicted constancy. The blood component seems to be more satisfactorily expressed as a function of the fat-free body mass than as a function of total body weight or of body surface area.

Curare-like compounds. I. A comparison of six compounds using inclined screen technic. O. D. PRIDDLE, JR. AND Y. T. OESTER (introduced by A. G. MULDER). *Dept. of Pharmacology, Stritch School of Medicine, Loyola Univ., Chicago, Ill.*

D-tubocurarine chloride, and 5 other compounds which have been reported to have curare-like activity, were compared in mice, using both intravenous and intraperitoneal routes of administration. LD_{50}, paralysis (PD_{50}) and time for the onset of paralysis were obtained. The six compounds used were *d*-tubocurarine chloride (d. t. c.), dibrommethylate of 1,4-bis-(diethylaminomethyl)-naphthaline (C-9909), dihydro-beta-erythroidine (d. b. e.), *d*-tubocurarine dimethyl ether chloride (Tu 42–4), dimethyl ether of *d*-tubocurarine iodide (d. d. t. i.), and decamethonium iodide (C-10). An inclined screen was used to indicate the onset of paralysis. LD_{50} values in mg/kg. in mice, using intravenous administration, were: d. t. c.—0.15; Tu 42–4—0.25; d. d. t. i.—0.25; C-10—0.81; C-9909—1.70; d. b. e.—1.93. However the LD_{50} values in mg/kg. in mice using intraperitoneal administration were: d. t. c.—0.456; d. d. t. i.—0.65; Tu 42–4—1.48; C-9909—2.5; C-10—2.65; d. b. e.—3.75. It is noted from the above comparative data that on a mg/kg. basis, d. t. c. is the most lethal, and d. b. e. the least lethal curarizing agent of the 6 used. It was found that in mice injected intraperitoneally, the time for the onset of paralysis as indicated in the following series, could not be correlated with the LD_{50} series above, since d. d. t. i.—2.6 minutes; Tu 42–4—3.5 minutes; C-10—3.6 minutes; d. t. c.—3.9 minutes; C-9909—4.2 minutes; d. b. e.—4.3 minutes. The time of onset of paralysis in intravenously injected mice was so rapid that accurate timing could not be recorded. The indices of therapeutic safety were of the following order: Tu 42–4—1.190; C-9909—1.176; d. d. t. i.—0.840; C-10—0.450; d. t. c.—0.114; d. b. e.—0.080. PD_{50} comparisons were also made and were not found to be directly related to the LD_{50} doses on a mg/kg. basis.

Propagation in non-striated muscles of invertebrates. C. L. PROSSER, H. J. CURTIS AND D. E. TRAVIS.* *Marine Biological Lab., Woods Hole, Mass.*

Retractor muscles of the proboscis of Phascolosoma showed two types of action potential as recorded with a d.c. amplifier. The 'fast' component was propagated at 1.6 m/second, it had a sharp threshold, was not graded, fatigued rapidly on repetitive stimulation, had absolute and relative refractory periods of 15 and 50 msec., respectively, and was associated with rapid initial shortening. The 'slow' component was propagated at 0.3 m/second, was markedly graded, became diffuse with increasing conduction distance, it facilitated greatly and because of this facilitation showed no apparent refractory period, it showed little fatigue and was associated with maintained contraction. The fast component was readily eliminated by local injury or KCl. Both were conducted in either direction in the muscle. The slow component is neither an after-potential nor an e.p.p.-like potential; there seem to be two separate conduction systems in the muscle fibers. Both types of potential were also found in lantern retractors of Thyone, the fast type alone was found in body wall muscle of Thyone and the slow type alone in byssus retractors of Mytilus.

Excretion of chloride and water in normal man. THEODORE N. PULLMAN AND WILLIAM W. MCCLURE (introduced by ALF S. ALVING). *Univ. of Chicago, Dept. of Medicine, Chicago, Ill.*

Excretion of chloride and water has been studied during 122 clearance periods in 6 normal men under a wide range of urine flows, serum chloride concen-

trations, and glomerular filtration rates. The subjects also received NaSCN, but preliminary data indicate that similar relations obtain without it. The fraction of filtered chloride reabsorbed was linearly related to the fraction of filtered water undergoing tubular reabsorption. This linearity was preserved under widely divergent conditions. By varying the serum chloride concentration, however, the slope of this line could be made to vary between almost zero and 2.14. Low slopes were associated with low serum chlorides and high slopes with high serum chlorides. This circumstance, obtained in man under water diuresis, parallels findings of Cizek and Holmes in the dog under osmotic diuresis. That the concentration of serum chloride is not the sole factor determining the slope of the above curves, is indicated by the fact that $NaHCO_3$ infusion increased these slopes, even though maintaining hypochloremia. In order to evaluate the reliability of different variables in predicting the inclinations of such curves, the slope of a line connecting the point of 100% chloride and water reabsorption and the point indicating the observed respective reabsorptive fractions, was calculated. This value is hyperbolically related to the urine chloride concentration, and one can be predicted from the other with considerable precision. Studies are under way to extend these observations to subjects not receiving NaSCN and to define an analogous relation in the case of sodium reabsorption.

Acute intestinal radiation death. HENRY QUASTLER (introduced by R. E. JOHNSON). *Univ. of Illinois, Urbana.*

There are several distinct types of radiation death. The form we call 'acute intestinal radiation death' occurs, in mice, 3.5 ± 0.3 days after doses from 1200–12,000 r. To produce this particular injury, it is necessary and sufficient to irradiate any large portion of the small intestine. Death is preceded by denudation of the villi, and seems to be toxic (bacterial invasion is not a factor in this type of radiation death). The stripping of the villi is due less to injury than to normal moulting in the absence of replacements from the crypts; the cell population decreases evenly after irradiation, but the remaining cells spread out so that the submucosa is not exposed until almost all cells are gone. Thus, the invariance of life expectancy over a wide range of doses is traced to the normal life cycle of intestinal epithelium. Radiation injuries of two kinds block the production of cells in the crypts. Effects on the cellular level are degeneration of dividing cells and arrest of mitotic activity. These effects are conspicuous and early, can be produced by small doses (even without direct irradiation), but are neither lasting nor critical. Other effects operate on the genetic level: the wasting of cells in spite of mitotic activity is attributed to the well-known genetic death after mitosis. The following analysis of acute intestinal radiation death is proposed: genetic effects cause degeneration of newly formed cells in the crypts; this leads to depletion of the epithelial lining, exposure of the submucosa, toxicosis, and death.

Accurate registration of intra-abdominal pressures. J. P. QUIGLEY, D. A. BRODY* AND W. C. LANDOLINA.* *Dept. of Physiology, Univ. of Tennessee, School of Biological Sciences, Memphis.*

Intraperitoneal pressures have been measured accurately, continuously and rather physiologically in anesthetized and unanesthetized dogs. A soft, flexible, saline-filled plastic catheter (external diam. 1.25 mm.) was introduced through the abdominal wall and connected with a Statham, Model P23-15G-2000 pressure transducer. Physiological saline solution under relatively high pressure was introduced into the transducer chamber through a fine constriction at the rate of 3–5 cc/hr., thus, sufficient volume to 'drive' the transducer without significantly altering its static or dynamic response was provided. The transducer output fed a carrier-type bridge amplifier which operated a Sanborn recorder through a direct-coupled amplifier. The hypothesis that intraperitoneal contents exhibit hydrostatic properties similar to a fluid was confirmed by comparing intra-abdominal pressures with pressures from a saline phantom outside the body: *1*) With animal in the horizontal position, the catheter tip was withdrawn in one-centimeter steps from an initial depth of 8 cm. *2*) with the dog in a vertical position, intraperitoneal pressures were recorded at various abdominal levels. Respiratory movements produced changes in intraperitoneal pressure near the xyphoid process of 2–3 cm. of water. The changes were *a*) entirely above the basal pressure, or *b*) a negative deflection immediately preceding and following the positive deflection. In the latter circumstance a characteristic asynchrony of the respiratory movements was noted in which thoracic expansion preceded apparent movement of the diaphragm.

An in vivo method for determination of tonus changes of pyloric sphincter. J. P. QUIGLEY, H. LOUCKES* and D. A. BRODY.* *Dept. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

Behavior of the pyloric sphincter of well-trained, unanesthetized dogs was studied by a modification of the inductographic technic of Brody and Quigley (*J. Lab. & Clin. Med.* 29: 863, 1944). Three pairs of coils were used independently for the determination of the magnitude of relaxation and contraction of the pyloric sphincter.

One pair, sutured diametrically opposite each other on the pyloric sphincter of the test animal, recorded sphincter activity. A second pair with a fixed separation, permanently connected into the apparatus, checked the constancy of the apparatus. A third pair which could be separated in steps accurate to 0.1 mm. calibrated the apparatus in terms of absolute coil separation. For a given galvanometer deflection, the coil separation was 2.5% greater (correction factor 1.02) when separated by air (calibration) than when separated by tissue (animal). Contrary to the usual teaching, the tonus (degree of relaxation) of the pyloric sphincter in well-trained animals was remarkably constant. During the one-hour period following ingestion of a meal, sphincter tone gradually decreased to permit a coil separation of an additional 1–2 mm. Maximal contractions also increased gradually during evacuation, distance between coils resulting from a contraction approximated 6 mm. before and 8 mm. one hour after feeding. Theoretical analysis and also incidental observations apparently justifies our assumption that sphincter external diameter (distance between coils) accurately reflects the sphincter lumen diameter.

Sympathomimetic and DCA pressor effects weakened by sodium-poor diet. W. RAAB, R. J. HUMPHREYS* AND E. LEPESCHKIN.* *Univ. of Vermont Med. College, Burlington.*

In 4 hypertensive individuals, the pressor effect of intravenous infusions of epinephrine and l-norepinephrine (0.1–0.2 γ/kg/min. for 5 minutes each) was tested before, during and after 6- to 10-week periods of a practically sodium-free rice fruit diet (200–400 mg. Na). In all cases, there was a slight to moderate decrease of the resting systolic blood pressure and in 3 cases also of the diastolic pressure. During the sodium-poor regime, the systolic pressure maxima, elicited by epinephrine and norepinephrine, were markedly lowered in 3 cases and slightly in one. The diastolic maxima were lowered similarly but to a somewhat lesser degree. After resumption of a normal diet, the epinephrine- and nor-epinephrine-induced pressure maxima increased again gradually. DCA, which otherwise regularly enhances the pressor action of both epinephrine and nor-epinephrine (*J. Clin. Investigation* 29: 1397, 1950) failed to produce this effect in 3 cases during the sodium-poor regime. It is tentatively suggested that DCA-like cortical steroids maintain and, if present in excess, augment the blood pressure level by sensitizing the cardiovascular contractile elements for adrenergic stimuli, through intracellular deposition of sodium (Darrow, Robertson) and through the effect of the latter on the cellular electric membrane potential (Fleckenstein). (Supported by a grant from the National Heart Institute.)

Respiratory gas exchange following tilting from standing to supine position. HERMANN RAHN AND RICHARD AMENT.* *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

These observations describe the rather dramatic changes in respiratory gas exchange which occur within a 2-minute period after a subject is tilted from the passive standing position to the supine position. The ventilation is increased above that of the standing value. This is of particular interest since at the same time the alveolar pO_2 falls on the average 14 mm. and the CO_2 rises in spite of the brief hyperventilation. These facts suggest that the ventilation actually lags behind the sudden demands made by the blood. Measurements of oxygen consumption show that 340 cc. are taken up in addition to the usual amount required during the supine position. The CO_2 output also increases. The CO_2/O_2 exchange ratio is .65. This increased metabolic rate can only in part be explained by the return of pooled venous blood low in oxygen. The remainder is probably a paying off of the tissue oxygen debt developed during passive standing.

Clearance of potassium esterone sulfate from the blood following intravenous injection. A. E. RAKOFF AND MONA L. GROSS (introduced by I. J. PINCUS). *Jefferson Med. College, Philadelphia, Pa.*

Potassium estrone sulfate in aqueous solution (20 mg/cc.) was injected intravenously in 10 normal young adults, 7 women and 3 men. Blood was taken for estrogen determination prior to injection and at 5 minutes, 1 hour, 5 hours, and 24 hours after injection. Whole serum was assayed for estrogen in castrate mice using the technic of Fluhmann. Following the injection of 20 mg. of potassium estrone sulfate, high serum estrogen values were obtained at 5 minutes (200–400 MU/100 cc.); at 1 hour the values had dropped markedly (less than 20 to 80); at 5 hours only small amounts were generally found (less than 10–20), while at 24 hours the serum estrogen had returned to about pre-injection levels. The results were similar in both sexes. Following the injection of 200 mg. of potassium estrone sulfate, very much higher blood levels were obtained at 5 minutes but there was nearly complete clearance in 5 hours. Similar curves were obtained in 2 rabbits. The hormone in the blood was present predominately in the free form and appeared rapidly in the urine. The blood estrogen clearance was delayed in some patients with liver disease. Normal values were obtained in various types of amenorrhea. This test procedure was more satisfactory than clearance tests with intramuscular injections of estrogen because of the higher blood levels attained and the more rapid

clearance. There were no immediate or delayed untoward effects.

Relation of nor-epinephrine and other autonomic agents to vascular collapse of stressed adrenalectomized animal. E. R. RAMEY,* M. S. GOLDSTEIN,* I. FRITZ* AND RACHMIEL LEVINE. *Dept. of Metabolic and Endocrine Research, Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

Previous work from this laboratory has shown that the poor work performance of the adrenalectomized animal is due to a fall in blood pressure induced by the stress of muscle stimulation. The present work relates this vascular collapse to the autonomic vasoconstrictor mechanisms, by demonstrating that: *1*) the DCA-treated adrenalectomized dog shows an abnormally low pressor response to nor-epinephrine; *2*) this insensitivity to nor-epinephrine becomes progressively more marked as the stress continues; *3*) ACE, but not DCA glucoside, produces a rapid rise in blood pressure in the stressed animal, when given with the ineffective nor-epinephrine; *4*) atropine significantly inhibits the blood pressure fall during stress. These data as well as observations on the reactivity of the blood vessels of the rat mesoappendix during stress indicate that the C_{11}-oxysteroids act locally on the small splanchnic vessels to sensitize them to the action of nor-epinephrine. These steroids also enable the blood vessels to recover from the damaging effects of large doses of nor-epinephrine. The relation of the autonomic effector agents to the mechanism of the vascular collapse of adrenal insufficiency will be discussed.

Dermatomal recruitment of sweating in response to heat. W. C. RANDALL, A. B. HERTZMAN AND H. E. EDERSTROM. *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Regional sweating activities were studied by the desiccating capsule and the iodine-starch-paper techniques on nude subjects exposed to various thermal stresses. During equilibrium studies with the subject in a constant environment sweating is usually limited to the more distal portion of the lower extremities. However, with increasingly warmer environments, sudomotor activity spreads up the leg to involve the trunk and finally the arm and face, in a fairly regular sequence. During exposure to a rapidly rising ambient temperature (from about 25°C.–40°C.), or sudden exposure to a high temperature (approximately 40°C.) essentially the same sequence in initiation of sweating was observed. Sweating was characteristically cyclic, the peaks of sweating becoming successively higher with rising environmental temperature. Adjacent dermatomes frequently showed marked differences in recruitment of sweat glands, and marked sweating was commonly observed on the lower extremity without the slightest trace of sweating on the upper part of the body. Particularly interesting was the reciprocal relation frequently observed on the hands and feet, where cyclic sweating progressively increased on the dorsal surfaces and decreased on the palmar and plantar surfaces with rising temperatures. (Aided by contract with the Air Materiel Command, Army Air Forces).

Effect of renin tachyphylaxis on survival time after severe hemorrhage. G. CARL RAU (introduced by B. A. WESTFALL). *Depts. of Physiology and Pharmacology, Univ. of Missouri Med. School, Columbia.*

Ten dogs anesthetized with sodium pentobarbital (32.5 mg/kg. intraperitoneally) were rendered completely tachyphylactic by repeated injections of 2 Goldblatt units of renin given in the left femoral vein at 5- to 8-minute intervals. The dogs then were hemorrhaged from the right femoral artery at a rate of 100 cc. every 3–5 minutes until 40% of the total blood volume was removed. Ten control dogs were anesthetized as described above and bled at the same rate until a 40% hemorrhage was produced. These dogs received no renin injections. All 10 of the tachyphylactic dogs died approximately one hour and a half (mean 90 minutes) after hemorrhage. However, none of the 10 control dogs died after hemorrhage, but were killed 3–7 hours later to terminate the experiments. These findings suggest that renin may function in dogs, at least as a protective agent against hemorrhage stress.

Abolition of induced positive synovial potentials in the dog by cortisone. C. I. REED AND NORMAN R. JOSEPH.* *Univ. of Illinois College of Medicine, Chicago.*

Following our observations that both ACTH and cortisone will reduce the high positive synovial potentials found in rheumatoid arthritis, it seemed desirable to determine whether high induced positive synovial potentials in normal dogs might be similarly affected. Glucosamine, PABA, PASA, guanine and adenine when employed by technics previously described produced positive potentials of 20–40 mv. Intramuscular injection of cortisone acetate after 30 minutes reduced or abolished these high positive potentials. Chronic treatment with cortisone acetate over a period of days resulted in a striking reduction for the first 2 or 3 days with subsequent progressive loss of effectiveness complete in 10–15 days. Thus both acute and chronic administration of cortisone abolished positive potentials induced by this series of substances but did not affect similar high positive potentials

induced by cupric ions, iodide ion, malonate, iodoacetate and cobalt.

Eosinopenic response of mice to ACTH protein and peptide. W. O. REINHARDT, G. F. HUNGERFORD* AND CHOH HAO LI. *Depts. of Anatomy and Biochemistry, Univ. of California, Berkeley.*

The blood eosinophil level in unfasted young adult male Swiss albino mice maintained under uniform environmental and experimental conditions averaged 497 ± 17 cells/mm.^{3} in 491 determinations, as determined by the direct counting procedure employing acetone phloxine diluting fluid. Injection of physiological saline solution IP or SQ produced, in the 4-hour test, a fall of 17 ± 5% in the eosinophil level in 80 mice. Assay of 9 ACTH protein and 11 ACTH peptide fractions at the 20-gamma level in groups of 6 mice per fraction gave average falls in the eosinophil level of 45% for each of the two types of preparations as compared with saline injected controls. Four peptide and protein fractions with distinct ascorbic acid lowering activity tested in the same manner produced a 28% increase in the eosinophil level. Four fractions without ascorbic acid activity produced a negligible eosinopenia, whereas one fraction produced an eosinophilia, and one peptide fraction without ascorbic acid activity produced a distinct eosinopenia. Within the conditions of these experiments, there was no obvious close correlation between the eosinopenic activity of either protein or peptide fractions as tested above, and the degree of ascorbic acid lowering activity. These results suggest that the eosinophil response of normal male mice to injected protein or peptide ACTH provides only a rough guide to the ascorbic acid potency of a given preparation. On the other hand, equivalent amounts of protein or peptide with different ascorbic acid lowering activity assay levels, may produce equivalent eosinopenic responses.

An index to available reserve bleeding volume. JOHN W. REMINGTON. *Dept. of Physiology, Med. College of Georgia, Augusta.*

The lethal bleeding volumes of presumably normal dogs may vary from 20 to 55 cc/kg. Comparisons of local fluid loss after trauma to an average lethal hemorrhage value is therefore always uncertain. Isuprel gives a maximal cardiac stimulation, with a large cardiac output increase at the expense of venous and cardiac reservoirs. It is also a vasodilator. If the mobilized blood can more than fill the enlarged peripheral bed, the high flow is sustained. This flow level should vary with the effective blood volume. Dogs were bled by 5 cc/kg. every 10 minutes, isuprel responses being determined after each bleeding. A definite correlation between flows after isuprel and the remaining bleeding volume is evident. When the former is used to predict the bleeding volume, the average error is ±5 cc/kg. Individual cases may err as much as ±12.5 cc/kg. Five dogs given 20 mg/kg. Dibenamine had an average bleeding volume of 25 cc/kg. The initial isuprel prediction was 32 cc/kg., but after the first 5-cc. bleeding this fell to 19 cc/kg., and subsequent agreement was good. Seven dogs, with initial isuprel indexes of 40 cc/kg. were given muscle trauma, producing fatal shock in 6. Post-trauma isuprel indexes average 15 cc/kg., which does not usually predict a fatal outcome in bled dogs. Five dogs with both kidneys tied showed a suggestively small lethal bleeding volume of 33 cc/kg. Control isuprel indexes average 36 cc/kg., however, as against the 41 cc/kg. of controls.

Lamprey hemoglobin. AUSTEN RIGGS* AND GEORGE WALD. *Biological Labs. of Harvard Univ., Cambridge, Mass.*

The lampreys, cyclostomes representing the most primitive living vertebrates, possess a blood hemoglobin with unique properties. Other vertebrate blood hemoglobins have molecular weights of about 68,000, lamprey hemoglobin about 17,000, like muscle hemoglobin (SVEDBERG AND HEDENIUS). Other vertebrate blood and muscle hemoglobins are isoelectric at pH 6.7–7, lamprey hemoglobin at pH 5.7, like invertebrate hemoglobins (ROCHE AND FONTAINE). We have measured the oxygen equilibrium of the hemoglobin of the sea lamprey, *Petromyzon marinus*. The equilibrium curve is a rectangular hyperbola, suggesting the presence of a single heme; n in the Hill equation is 1.0. This is the only vertebrate blood hemoglobin known to possess this property. Myohemoglobin and the invertebrate hemoglobins which have hyperbolic oxygen equilibria, also have high affinities for oxygen, and small or negligible Bohr effects. Consequently, they do not discharge their oxygen readily, and are adapted for oxygen storage rather than transport. In the lamprey the oxygen equilibrium curve, though hyperbolic , lies at high oxygen tensions. The pressure of oxygen at which the hemoglobin is half-saturated (p_{50}) is about 19 mm. Hg at pH 6.8 (20°C.), compared with a p_{50} of about 0.6 mm. in mammalian muscle hemoglobin (19°C.). Lamprey hemoglobin also exhibits a large Bohr effect, the p_{50} changing from about 10 mm. Hg at pH 7.4 to about 70 mm. at pH 6.3. The pH of lamprey blood serum is about 6.6. These are adaptations for oxygen transport in an active animal. With its low oxygen affinity, lamprey hemoglobin unloads oxygen readily, and at high tensions that promote rapid diffusion into the tissues; while the Bohr effect helps to expel oxygen under venous conditions. (This research was supported in part by a grant to G. W. by the Medical Sciences Division of the Office of Naval Research.)

Further observations concerning electrokymographic records from the dog's heart. GORDON C. RING. *Dept. of Physiology, Temple Med. School, Philadelphia, Pa.*

A method requiring no surgery which would accurately record the contour of the inward movements of the ventricles during ejection would make possible a great increase in our knowledge of cardiac function. The electrokymograph (EKY) provides records of these movements but usually these graphs are seriously distorted by positional movements of the heart. These distortions can be reduced by recording densograms rather than border movements and by choosing an area near the middle of the ventricular silhouette where the portion of the heart moving under the recorder is of the same thickness as that which it replaces. A further improvement is obtained if the animal is so placed that the movements of the heart are toward or away from the EKY recorder. A study of the positional movements of the dog heart have shown that early in systole the apex moves sternally and the base spinally. Late in the cycle both base and apex appear to move toward the spine. In large dogs, if the animal is turned in an oblique position and one records 3 cm. up from apex and 4 cm. in from sternal edge, then a graph is obtained having systolic contours which appear to represent the true inward movements.

Effects of cortisone and desoxycorticosterone acetate in adrenalectomized dogs. KATHLEEN E. ROBERTS (introduced by WILLIAM D. LOTSPEICH). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

Intravenous administration of cortisone in dosage of 1.5 mg/kg. to adrenalectomized dogs, at a time when the animals were without maintenance therapy but not yet in a state of insufficiency, resulted in a marked decrease in sodium excretion and an increase in renal plasma flow. No consistent changes in glomerular filtration rate have been observed. Similar results were obtained following the intravenous administration of emulsified desoxycorticosterone acetate in dosage of 80 μg/kg., i.e. a marked decrease in sodium excretion and a rise in renal plasma flow. The injection of cortisone, in dosage of 1.5 mg/kg., 30–45 minutes following the administration of desoxycorticosterone acetate, in dosage of 80 μg/kg., resulted in a decreased sodium excretion and a rise in renal plasma flow similar to the changes seen when each agent was given alone. Under the conditions of these experiments, no antagonism between cortisone and desoxycorticosterone on sodium excretion was apparent; both agents, when given alone, or in combination, resulting in a decreased sodium excretion, a rise in renal plasma flow and no consistent change in glomerular filtration rate. (Aided by a grant from the American Heart Association.)

Inhibition of histamine induced gastric acid secretion by irrigation of antihistaminics into gastric pouches in dogs. CHARLOTTE ROBERTSON (introduced by R. GRANT). *Dept. of Clin. Science, Univ. of Illinois College of Medicine, Chicago.*

None of the available antihistaminics inhibit histamine-induced gastric secretion even when maximal tolerated doses are given parenterally. In order to test the possibility that a very high local concentration in the gastric mucosa might be effective we have given antihistaminics by irrigating solutions of them into gastric pouches (vagally denervated) of dogs receiving 0.04 mg. of histamine diphosphate subcutaneously every 10 minutes. Solutions containing 50 mg/cc. of Neoantergan or of Pyribenzamine irrigated into the pouches for one-half hour cause cessation of acid secretion for periods of 0.5–4 or more hours. The mechanism of this effect and the implications of it in regard to the physiological control of gastric acid secretion are being studied.

Sensory mechanisms in hippocampus, cingulate gyrus and cerebellum of the cat. FRANKLIN ROBINSON AND MARGARET A. LENNOX (introduced by DONALD H. BARRON). *Lab. of Physiology and Dept. of Psychiatry and Mental Hygiene, Yale Univ. School of Medicine, New Haven, Conn.*

The hippocampal formation, cingulate gyrus and cerebellum were studied in relation to functional participation in afferent receiving mechanisms. By means of evoked potential technique in the cat, electrical responses were recorded from known cell stations and pathways of these afferent systems after direct operative exposure or by means of Horsely-Clark technique. Electrical potentials in response to click stimuli were recorded chiefly from the anterior portion of the hippocampus and these were characterized by relatively brief latency, short duration of response and low amplitude as compared with potential patterns from known brainstem and subcortical pathways. Potential changes were recorded from the surface and depth of the hippocampal formation following single shock stimuli to the optic nerve. These responses were obtained from the anterior portion including the region of the amygdaloid nucleus and were characterized by short latency, low amplitude and short duration of response. After single shock stimuli to the proximal stump of the sciatic nerve, similar responses were recorded from the surface of the exposed hippocampus predominantly over its mid-portion. Optic and acoustic responses of slightly longer latency than those recorded from the hippocampus

were obtained from the posterior cingulate gyrus. Afferent representation in the cerebellum as described by others has been confirmed. These experimental observations are consistent with theoretical assumptions of Papez and MacLean as to representation of various sensory modalities in the hippocampal formation.

Effect of increased intratracheal tension on pulmonary and systemic arterial pressures in absence of a ventricular septum. S. RODBARD AND A. SAGAN.* *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

In animals with normal four-chambered hearts, increased intratracheal pressure (produced by inflating the lungs in open-chested dogs, rabbits or chicks) raised the resistance to flow through the pulmonary capillaries. Pulmonary arterial pressure was thereby increased. The reduced flow to the left ventricle resulted in a fall in systemic arterial pressure. When the intratracheal pressure was raised in animals without ventricular septa, as in turtles, systemic and pulmonary arterial pressures both increased. Thus an increase in intratracheal pressure of 20 mm. Hg was followed within 2–3 beats by an equal rise in the pulmonary and systemic pressures. This effect is seen in the intact animal or after removing the plastron; it is independent of central nervous control since it occurred in the pithed preparation. The effect is probably due to an increased resistance to pulmonary blood flow, which shunts a greater proportion of ventricular output, to the systemic circuit. The heightened systemic flow increases the diastolic pressure and this rise is reflected in the pulmonary artery. Similar mechanisms probably account for the shunting of unoxygenated blood through ventricular septal defects to the systemic circuit with the production of cyanosis during the Valsalva maneuver, as during crying in children with congenital cardiovascular malformations. Variations in the intratracheal pressure, therefore, by their effect on the resistance to flow through the pulmonary vessels may be useful in the analysis of cyanotic or potentially cyanotic congenital cardiovascular lesions.

Impairment of alveolar ventilation during anesthesia in man. ALBERT ROOS AND JAMES G. GABBARD.* *Lab. of Thoracic Physiology, Depts. of Surgery and Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

Velocities of inspired and expired gases and pulmonary ventilation were continuously recorded by means of a pneumotachograph of low dead space (25 cc.) interposed in the standard anesthesia circuit. In this manner an evaluation of alveolar ventilation during the operative procedure was obtained. Frequent arterial blood samples were analyzed for oxygen saturation, plasma CO_2 content and pH. The effect of the following factors on alveolar ventilation was investigated: *1*) premedication, *2*) manual assistance of breathing, *3*) anesthetic agent, and *4*) open thorax. Unassisted ether anesthesia with an open thorax invariably produced inadequate alveolar ventilation and respiratory acidosis. These deviations could be remedied by manual assistance. However, they were often present as well before the chest was opened, both in the supine and lateral positions. In those non-thoracic procedures where lighter ether anesthesia was sufficient, adequate ventilation and normal blood values were obtained without assistance to breathing. A quantitative correlation was shown between the degree of inadequacy of ventilation and the magnitude of the respiratory acidosis. The high pCO_2 values obtained under these conditions indicate a greatly decreased sensitivity of the respiratory center to CO_2. Morphine premedication seemed to have little further depressant effect. These data were compared with results using other anesthetic agents.

A statistical model for neural responses to acoustic clicks. WALTER A. ROSENBLITH AND WILLIAM J. MCGILL (introduced by S. S. STEVENS). *Psycho-Acoustic Lab., Harvard Univ., Cambridge, Mass.*

When the response to acoustic clicks is recorded from the round window of cats the amplitude of the first neural component (N_1) is, at least for weak clicks, a monotonic function of stimulus intensity. If we assume that this amplitude is proportional to the number of independent neural units firing at a given time, the neural response can be related to the intensity of the click stimulus by a simple probability function. The function relating amplitude of N_1 to intensity becomes specifiable in terms of two statistical parameters: the mean and the standard deviation of a normal distribution. Factors that affect the click response are presumed to act upon these parameters. If two consecutive clicks are delivered to the same ear, our model predicts that the amplitude of N_1 for the second click is a function of the intensity of the first click and of the time interval between the clicks. Experimentally determined recovery functions (which extend over more than 100 msec.) are in satisfactory agreement with predicted values. This probability model can be modified to yield predictions for click experiments involving multiple clicks, masking, fatigue and temperature change.

Response of human athyreotics to levorotatory thyroxine, administered orally. IRA ROSENBLUM (introduced by WILLIAM T. SALTER). *Dept. of*

Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.

Levorotatory thyroxine has been administered orally in tablet form to *a*) several adult human patients whose thyroid had been destroyed with radioiodine, I^{131}, and *b*) to a cretin child. These individuals initially showed the classical clinical and laboratory findings characteristic of complete athyreosis. They were treated with graded doses of L-thyroxine; and all made a striking clinical response. At monthly intervals the daily-dosage was increased by increments of 0.1 mg. for adults and 0.05 mg. for the cretin child. After a steady state had been attained for each dosage level, the serum 'hormonal' iodine was determined. This value rose progressively from the initial value of about 1 μg. %; and reached normal levels (about 5 μg. %) when the daily dosage was 0.3 mg. for adults and 0.15 mg. for the 5-year-old cretin. For adults, the dosage-response (μg. % of serum iodine/mg. of drug) showed a slope value of approximately 12. Per square meter of surface area this average value became 19 μg. %/mg. of drug (i.e. daily dose at equilibrium). When added *in vitro* to human serum, the L-thyroxine was *not* protein-bound. An hour after the patient ingested the drug, however, the protein-bound iodine rose temporarily to hyperthyroid levels (e.g. 16 μg. %) during the post-absorptive phase.

Discrimination of interaural time-differences at auditory cortex. MARK R. ROSENZWEIG (introduced by J. C. R. LICKLIDER). *Psycho-Acoustic Lab., Harvard Univ., Cambridge, Mass.*

Localizing the source of a sound depends chiefly upon the difference in time-of-arrival of the sound at the two ears. In order to use this clue, the auditory nervous system must discriminate interaural time differences which are only fractions of a millisecond in duration. The experiments to be reported here were performed to see whether such small interaural time-differences are represented in the electrical activity of the auditory cortex. Electrical activity was recorded from the cortex of anesthetized cats. The two ears of the animal were stimulated independently with separate earphones, and the time-difference between click stimuli at the two ears was varied systematically. When only one ear is stimulated, responses appear in the auditory areas of both cerebral hemispheres, but the response is usually larger in amplitude at the hemisphere contralateral to the stimulated ear. When both ears are stimulated and there is an interaural time-difference, the response tends to be larger at the hemisphere contralateral to the ear which receives the prior stimulus. The difference between the amplitudes of the responses at the two hemispheres can be shown especially clearly when the responses are recorded differentially between the two hemispheres. In this way, time-differences of less than 0.1 msec. can be demonstrated to be effective in altering the relative amplitudes of the responses at the two hemispheres. Hypotheses concerning the neural mechanisms of this discrimination will be presented.

Effect of 3-β-aminoethylpyrazole dihydrochloride on gastric secretion in dogs. C. E. ROSIERE* AND M. I. GROSSMAN. *Dept. of Clin. Science, Univ. of Illinois College of Medicine, Chicago.*

With the hope of finding an inhibitor of gastric secretion we have been screening compounds closely related to histamine; 41 have been studied to date. No inhibitors have been found but one compound, 3-β-aminoethylpyrazole (compound XXIV), possesses the remarkable property of stimulating gastric acid secretion although (as reported by Lee and Jones) it is apparently devoid of any other histamine-like action such as vasodepression and contraction of smooth muscle. In 4 dogs with pouches of the entire stomach a dose response curve for histamine was made by giving graded doses subcutaneously every ten minutes and determining the acid output when a plateau of secretion had been attained. A similar dose response curve was made for XXIV. The average dose of histamine required to produce 50% of the maximal rate of acid secretion was 0.073 mg. every 10 minutes (calculated as histamine base), the corresponding value for XXIV was 4.9 mg., giving a potency ratio of 67 to 1. The gastric juice secreted in response to XXIV has a low pepsin concentration like that obtained with histamine. The new drug was also given to a series of human subjects in dosages of 25, 50 and 100 mg. intramuscularly. Marked stimulation of acid secretion was observed. Significantly the subjects experienced no headache, flush, 'triple response' or any other side-effect although the stimulation of acid secretion was comparable to or greater than that obtained with 0.01 mg. histamine base per kg. The remarkable specificity for gastric acid secretion of this new compound indicates that it will be useful clinically in establishing the existence of achlorhydria as well as for physiological studies. (The compound XXIV used in this study was kindly supplied by Drs. Lee and Jones of the Lilly Research Laboratory.)

Methemoglobin reduction by the avian erythrocyte. HOWARD H. ROSTORFER, WILLIAM F. GEBER* AND HAROLD R. ONYETT.* *Dept. of Physiology, Indiana Univ., Bloomington, Ind.*

The oxidative metabolism of methemoglobinized avian and mammalian erythrocytes was measured *in vitro* by the 3-flask technique with Dixon-Keilin flasks during the process of methemoglobin reduction which was followed by Van Slyke analyses

of flasks similarly set up. Blood samples from dogs and ducks obtained by heart puncture were treated with either sodium nitrite or phenylhydrazine to form comparable amounts of methemoglobin and then the cells were washed four times with cold saline. The nitrite treated avian and mammalian cells reduced methemoglobin at the rates of 1.57 and 1.34 gm.%/hour respectively, and exhibited a concomitant increase in oxidative metabolism of 20 μl. O_2, a 60% increase, and 12 μl. O_2, a 10-fold increase, respectively above their washed cell controls. Neither type of cell reduced methemoglobin in the absence of substrate. In each the anaerobic reduction was equal to the aerobic reduction of methemoglobin. When phenylhydrazine (1–2 mg/ml. blood) was used, the avian cells in the presence of glucose showed a gradually increasing oxygen capacity for the first 2 hours while the mammalian cells exhibited only a gradual loss of capacity. The difference in capacities of the avian cells with and without glucose was 1.5 gm.%/hour, but the actual increase in capacity over the zero time level for the cells with glucose was less than .5 gm.%. These data suggest that the mechanism of methemoglobin reduction is essentially the same for avian and mammalian cells and that phenylhydrazine forms methemoglobin, but also attacks the oxidative enzymes and probably forms some inactive molecule besides methemoglobin from hemoglobin.

Reversal of cutaneous temperatures of digits of upper and lower extremities after extensive sympathectomy and its relation to basal metabolic rate. GRACE M. ROTH AND WINCHELL MCK. CRAIG.* *Sections on Physiology and Neurosurgery, Mayo Clinic, Rochester, Minn.*

Previously, Roth and Sheard reported the reversal of the cutaneous temperatures of the digits of the upper and lower extremities after extensive sympathectomy. These results indicated that the regulatory control of the elimination of heat in order that the temperatures of the body may remain practically constant at 98.6°F. had been shifted from the lower to the upper extremities. Maddoch and Coller demonstrated a simple linear relationship in the same individual between the skin temperatures of the great toe and the basal heat production per unit of surface area. Later Sheard and Williams and Roth and Sheard found a linear relationship which, however, seemed to be of a dual character. Although little or no correlation has existed between the skin temperatures of the fingers and the basal metabolic rate, the question arose as to whether a dual linear relationship might become apparent between the skin temperature of the fingers and the basal heat production when the control of the elimination of heat had been shifted from the lower to the upper extremities. Cutaneous temperatures of the fingers and toes and basal metabolic rates of 180 patients were determined before and after extensive bilateral sympathectomy under basal conditions in a constant temperature room maintained at 25.5°C. and a relative humidity of 40%. One hundred and twenty-five of these patients or 69.4% showed a reversal of the cutaneous temperatures of the fingers and toes whereas 55 patients or 30.6% showed no reversal. While the average skin temperatures of the fingers increased as the basal heat production per unit of surface area increased, no definite correlation could be established between the cutaneous temperatures of the fingers and the basal heat production in those patients who showed a reversal of the cutaneous temperature of the digits of the upper and lower extremities.

Caffeine potentiation of gastric secretory response to maximal histamine stimulation, or to electrical stimulation of vagus nerves. JAMES L. A. ROTH AND DAVID C. H. SUN.* *Dept. of Gastroenterology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

The effect of caffeine upon other stimuli to gastric secretion was investigated in cats anesthetized with ether and prepared with a gastric cannula. *Maximal* gastric secretory response to histamine was established according to the method of Grossman (*Am. J. Physiol.* 153: 242, 1948). A single intravenous injection of caffeine (5 mg. base/kg.) was superimposed upon the maximal response to continuous histamine stimulation. The free acid output was increased by 61% above that obtained in response to the same maximal dose of histamine alone. Control experiments showed that this dose of caffeine never was sufficient in itself to stimulate. The technique employed in electrical stimulation of the vagus nerves was a modification of that used by Uvnas (*Acta Physiol. Scandinav.* 4: Supp. 13, 1942). Control experiments with electrical stimulation alone showed that after the free acid output reached a plateau level for variable periods of time, it invariably was followed by a gradual decline to the basal level. After the response to electrical stimulation of the vagi had been constant at a plateau level for 30 minutes, a single dose of caffeine was injected intravenously and gastric juice collected for an additional 60 minutes while electrical stimulation continued. Caffeine, in a non-stimulating dose of 5 mg. base/kg., increased the free acid output by 67% above that obtained from vagal stimulation alone. These results suggest that such relatively small doses of caffeine potentiate the secretory response by acting as a 'catalyst' upon the intracellular enzyme systems involved in HCl formation. The sustained free acid response to caffeine, observed in the majority

of duodenal ulcer patients, may thus be explained on the basis of a conditioning influence of excessive vagus nerve activity.

Potential antigenicity of hyaluronidase preparations. LAURENCE W. ROTH AND IDA MAE SHEPPERD.* *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

With the increasing use of hyaluronidase for clinical conditions, its potentialities for inducing hypersensitive states must not be ignored. Hyaluronidase from 8 different sources (two commercial preparations in standard dose ampoules and 6 experimental lots, in bulk, from various manufacturers) was used in 55 animals. Each sample was administered intraperitoneally to a group of 4–10 guinea pigs following the method previously described (ROTH, RICHARDS AND SHEPPERD, *J. Lab. & Clin. Med.* 34: 531, 1949). Sensitizing doses, varying from 2.5 to 10 mg. (or one ampoule) per animal, were given 6 times in 2 weeks. One month later a challenging dose, in the same range as that used for sensitizing, was administered intravenously to each animal. One or more anaphylactic deaths occurred with each sample, and the remainder of the animals exhibited various non-fatal anaphylactic symptoms. Limited experiments with a few animals showed a significant incidence of cross-reactions when animals sensitized to one preparation were challenged with another, although there were no fatalities. Nonsensitized controls receiving the same amount used for challenging showed no symptoms, except with one sample which was of microbiological origin. This, in higher doses, caused delayed deaths totally unlike anaphylaxis, presumably due to the presence of a toxin which was not separable by further purification. A summary of these experiments indicates a strong possibility that antigenicity is inherent to the hyaluronidase molecule itself and is not necessarily due to the presence of other antigenic impurities, as implied by some authors.

Factors influencing the continuous recording of gastrointestinal pH in situ. RANDOLPH A. ROVELSTAD,* CHARLES A. OWEN, JR.* AND THOMAS B. MAGATH. *Div. of Clinical Labs., Mayo Clinic, Rochester, Minn.*

The inherent inaccuracy of the dye indicator methods of measuring gastric acidity and the discrepancies between the pH of gastric juice tested 'simultaneously' *in situ* and in aspirated samples led to further study of *in situ* measurements. A system was designed which permits circulation of electrolyte to the intragastric capillary contact of a calomel electrode. Comparing this method with a skin-reference system, it was found that in continuous tracings on 30 normal subjects the *in situ* pH was consistently about one-half pH unit lower (stomach) or higher (duodenum). These electropotential differences between the skin and alimentary tract proved to be more reliable than roentgenograms for determining passage of the electrode from the stomach to the duodenum, since the former was more negative by 44 mv. With both glass electrode and reference contact in the stomach, fluctuations of about 1 pH unit occurred; these may be attributed to diffusion, demarcation, action and colloid potentials. Fluctuations of about 5 pH units which occurred at a 10-per-minute rate could explain discrepancies between the *in situ* pH values and those of aspirated samples. After the injection of histamine, pH values were lower if the glass electrode was shielded to prevent mucosal contact. The lowest gastric pH recorded in normal subjects was 0.9, the highest duodenal value was 7.9. The dynamic nature of data obtained with the continuous-recording *in situ* glass electrode indicates that this procedure is a valuable experimental and clinical adjunct.

Pulmonary densogram in the dog. H. W. RUDEL (introduced by E. M. GREISHEIMER). *Dept. of Physiology, Temple Univ. School of Medicine, Philadelphia, Pa.*

Using the electrokymograph (EKY) to measure the x-ray density changes which occur in the lung fields during each cardiac cycle, a record is obtained which resembles an arterial pressure pulse. Some investigators have considered using these densograms in conjunction with the Valsalva maneuver to measure pulmonary arterial pressure. Since this method has been shown to be theoretically unsound, it was felt that a study of the contours of these densograms might offer some insight into the problem of their use in indirectly measuring pulmonary arterial pressure. Such a study has been undertaken but it became apparent from the outset that the pulmonary densogram differed from the pulmonary arterial pressure pulse in two major respects. First, the beginning of increased x-ray density (beginning of the volume increase) does not correspond to the beginning of the pressure rise. Secondly, the point of maximal x-ray density (maximal volume change) does not correspond to the peak systolic pressure. These discrepancies are variable depending upon the area of lung field studied and seem to be independent of the distance from the central artery. The variability of the contour of the densogram can in large part be explained by the effect of the positional movement of the heart changing the density of the lung field. This does not mean that the pulmonary densogram is of no value for the measurement of pulmonary arterial pressure since a relationship between pulmonary ar-

terial pulse pressure and the amplitude of the densogram can be established.

Comparison of specific gravities determined by weighing and copper sulfate methods. C. H. WILLIAM RUHE (introduced by T. K. KRUSE). *Dept. of Physiology and Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

A comparison was made of specific gravities by weighing and by the copper sulfate method on beef, dog and rabbit serum and blood samples. Weighing was done in a 50-ml. pycnometer, with all values corrected to 25°C. Copper solutions differing by 0.0005 were weighed after preparation. Seventy-three samples of whole serum and of serum diluted with 0.9% NaCl solution ranged in specific gravity from 1.0328–1.0173. In 71 samples, weighing values were higher than copper sulfate values by an average of 0.0007 with the greatest difference being 0.0014; 75% of the samples differed by 0.0005 or more. In one sample the values were identical and in one sample the copper sulfate was higher by 0.0003. Twenty-five samples of whole blood and of blood diluted with its own serum ranged from 1.0549–1.0448. With the blood samples no consistent difference was noted between the two methods. In 14 samples the weighing-values were higher, in 3 samples the values were identical and in 8 samples the copper sulfate values were higher. The greatest difference was 0.0007 and the weighing averaged higher than the copper sulfate by 0.0001.

Action of vitamin B_{12} and of liver extract on nitrogen balance of hyperthyroid rats. J. RUPP (introduced by F. R. MILLER). *Jefferson Med. College, Philadelphia, Pa.*

Considerable experimental evidence has accumulated during the past several years showing that the weight loss of hyperthyroid animals can be prevented, and even normal growth of such animals maintained, if vitamin B_{12} or liver preparations are administered. This effect is largely due to stimulation of appetite induced by the supplements, but it has been suggested that in addition food utilization is improved. We have performed experiments on force-fed rats fed a constant amount of a diet containing about 16% protein derived from yeast and milk powder. Neither vitamin B_{12} (6 γ/day) nor liver extract (0.4 U.S.P. U = 0.2 ml/day) caused any change of weight or of N excretion. Rats fed in this way and made hyperthyroid by daily injection of 150 γ of *d-l* thyroxine, lost weight and exhibited increased N excretion. When such thyroxine-treated rats were given vitamin B_{12}, the thyroxine induced weight loss was not changed, but the N loss was significantly decreased. Since it is known that the calorigenic effect of thyroxine is not counteracted by the vitamin and since the weight loss of the hyperthyroid rat was not influenced by B_{12} or liver extract, it would appear that the latter are N sparing at the expense of material other than protein.

Mechanics of ventricular contraction: a cinefluorographic study. ROBERT F. RUSHMER, NATHAN THAL* AND ALLEN YOUNG*. *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Changes in the size and configuration of the right and left ventricular cavities were studied by cinefluorographic angiocardiography in 20 anesthetized dogs in the left lateral reclining position. During projection of these motion picture films, ventricular filling appeared to occur more rapidly than systolic ejection. Planimetric measurements of the projected ventricular areas were made from tracings of successive motion picture frames showing several cardiac cycles in 8 animals. In most cases, filling of the ventricular chambers rapidly reached a plateau in early diastole which was relatively constant until the next systole. Since ventricular filling is virtually complete in early diastole, the rate of ventricular filling would impose little limitation on augmented cardiac output by tachycardia until the cycle length is markedly reduced. The left ventricular chamber resembles a cylinder with a conoid segment extending from the apical end. Reduction in ventricular area during systole was accomplished largely by a reduction in the diameter of the chamber which was relatively constant in successive cycles. More complete emptying of the ventricle was accomplished largely by additional shortening of the chamber. At the end of systole, residual blood was invariably present in both the right and left ventricular cavities, but the amount varied in different animals and in successive cycles in the same animal. The walls of both ventricles became thicker during systole indicating a greater reduction in the radius of the inner shells of myocardium than in the outer layers. Since circumference is directly related to radius, circumferentially arranged muscle fibers near the endocardium would shorten to a greater degree than those nearer the external surface. From anatomical and geometrical points of view, the right ventricle has a large surface area in relation to its capacity, suggesting considerable lability in volume in response to varying filling pressures, but a corresponding vulnerability to high outflow pressures. The left ventricle is adapted to high outflow pressures.

Statistical analysis of renal clearance by the dog. H. F. RUSSO, J. L. CIMINERA AND S. R. GASS (introduced by KARL H. BEYER). *Dept. of*

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A statistical analysis of normal renal clearances was performed on data accumulated over a period of 6 years using 31 female dogs. Measurements of renal function included glomerular filtration (GF), renal plasma flow (RPF), and *p*-aminohippurate (PAH) Tm, and arginine Tm. All values presented herein are as per m.^{2} of surface area. Analysis of 2045 creatinine clearances (GF) for interaction between dogs and years by the method of G. W. Snedecor, (*Statistical Methods*, 4th ed., Iowa State College Press, 1948) yielded a mean value and a standard deviation (S.D.) of 94 ± 9 cc/minute; 95% confidence limits = 94 ± 18 cc/minute. From 290 determinations, the mean value and S.D. of a single PAH-RPF determination on any given dog for any one occasion were 238 ± 32 cc/minute; 95% confidence limits = 238 ± 63 cc/minute. Although only 20 determinations of Tm_{PAH} spread over 7 dogs were available it was determined that the variations between dogs probably were not significant. The mean value and the S.D. were 16.46 ± 1.79 cc/minute; 95% confidence limits = 16.46 ± 3.87 cc/minute. Analysis of $Tm_{arginine}$ was made by the method of Mohanlal Ganguli, (*Sankhya*, The Indian Journal of Statistics, 5: 449, 1941). The mean and S.D. of a single determination on any given dog for any one occasion are 12.88 ± 1.66 cc/minute; 95% confidence limits are 12.88 ± 3.25 cc/minute.

Severe radiation injury observed in the erythrocyte sedimentation of dogs. G. A. Sacher, M. N. Swift and J. Gurian (introduced by H. M. Patt). *Argonne Natl. Lab., Chicago, Ill., and Naval Radiological Defense Lab., San Francisco, Calif.*

When erythrocyte sedimentation curves are plotted as logarithm of distance versus logarithm of time, the result is a line of little curvature for about two-thirds of the total distance. The slope and intercept of a fitted line provide two independent parameters of the sedimentation process. Data from x-rayed dogs were reanalyzed in this fashion. In dogs which received 200 r or less, the slope falls to a minimum between 10 and 20 days, and the intercept reaches a maximum at about the same time, then both return to normal levels. In dogs which received 300 r or more, a new pattern appears. Intercept rises to a maximum and subsides as for lower doses, whereas the slope falls for the first 10 days, then rises abruptly to a maximum at 20 days, falls again to low values, and finally rises to normal level. The phase of increased slope is seen almost always after single massive doses, occasionally after protracted exposures (injected radioisotopes), and not at all in dogs which received daily doses of x-rays throughout life. The observations suggest that the phenomenon is a positively accelerated function of dose and dose rate. The behavior at low dosages can be accounted for in terms of hematocrit values, but no correlates for the increase in slope at higher dosages are known. The empirical parameters can be tentatively related to parameters of the physical sedimentation process.

In vitro studies on adrenal ascorbic acid. M. Saffran, Margaret J. Bayliss and James L. Webb (introduced by R. A. Cleghorn). *Allan Memorial Inst., McGill Univ., Montreal, Quebec, Canada.*

Rat adrenal tissue preparations, suspended in Ringer media, lose ascorbic acid into the medium. The extent of the loss varies with the damage incurred during preparation of the tissues but a portion of the ascorbic acid always remains associated with the tissue. Thus, the loss of ascorbic acid from adrenal homogenates, slices, and whole glands does not exceed 95%, 75% and 25% respectively, indicating the presence of readily-diffusible and tightly-bound forms. The addition *in vitro* of high concentrations of Armour's ACTH results in a significantly greater loss of ascorbic acid from whole rat adrenals into the medium. ACTH also causes a significant increase in the oxygen consumption of whole rat adrenals. These observations on the effects of ACTH confirm the report of Tepperman (*Endocrinology* 47: 384, 1950), using dog adrenal slices. The respiration of whole adrenals is comparable to that of adrenal slices. In solution, ascorbic acid is protected by ACTH against destruction, as determined by the Roe and Keuther method. This has been shown to be due to the protein properties of ACTH.

Effectiveness of commercial hemolytic agents. A. P. St. Onge* and D. B. Dill. *Med. Div., Army Chemical Center, Md.*

Saponin and other hemolytic agents (such as Aerosol-OT) have many laboratory applications. The most familiar use is in the gasometric procedure for oxygen and carbon dioxide in blood. Peters and Van Slyke recommend the use of saponin Merck in making the neutral ferricyanide reagent. Recent studies indicate that some post-war saponins do not function with full satisfaction. Alternative hemolysins such as Aerosol-OT and soapwort extract have been proposed but no quantitative evaluation of commercially available saponins have been reported. In our first studies the oxygen content of goat blood was determined using the neutral ferricyanide reagent in the recommended concentration of 0.3%. All available saponins were employed. The following hemolysins were entirely satisfactory: Penick Saponin; Eastman Saponin, Lot No. P4764; E. Merck Saponin

(Darmstadt, Germany); a prewar sample of Merck and Company (Rahway) Lot No. G-31331; Amend experimental sample; and American Cyanamid Aerosol-OT. Unsatisfactory saponins were J. T. Baker, Lot No. 1.2844, Lot No. 1.1144, Lot No. 72945 and Lot No. 2.2044; Amend Drug Company, Lot No. C48969; and Fisher Scientific Company Lot No. S-33, Lot No. J-97 and Lot No. 472977. Further studies on human blood agreed with the above results with the exception of the new Amend saponin. E. Merck Saponin (Darmstadt, Germany) and a British Drug House sample were satisfactory in a concentration of 0.1%. Aerosol OT was satisfactory in concentrations as low as 0.05%.

Influence of estrogen on Hooker-Forbes assay for progesterone. H. A. SALHANICK,* A. G. OLSEN* AND F. L. HISAW. *Dept. of Biological Chemistry, Univ. of Utah School of Medicine, Salt Lake City, and Biological Labs., Harvard Univ., Cambridge, Mass.*

Hooker and Forbes have described a microbioassay which detects as little as 0.33 μg. progesterone/ml. The technic involves the injection of 0.0006 ml. of the test sample into the ligated uterus of a mouse castrated for 16 days. The criterion of a positive result is the conversion 48 hours post-injection of the stromal nuclei from the castrate condition to enlarged, oval nuclei with fine chromatin granules and conspicuous nucleoli. We sometimes obtained 'false negatives' when using this method in studying the progesterone content of monkey and rat sera. In these instances considerable amounts of estrogen were suspected to be present. To determine whether or not estrogen had interfered with the response of the stromal nuclei to progesterone, we assayed test solutions of sesame oil and mammalian Ringer's containing constant amounts of progesterone (2 and 5 μg/ml.) and graded amounts of alpha-estradiol (0.001 to 10 μg/ml.). While 2 and 5 μg. progesterone/ml. always gave positive responses, solutions containing estradiol and progesterone in ratios of 1/200 or greater gave negative responses. Supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.)

Triphosphopyridine nucleotidase. D. R. SANADI (introduced by G. LEPAGE). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

The enzyme from pig kidney which converts triphosphopyridine nucleotide (TPN) to diphosphopyridine nucleotide (DPN) by splitting off a molecule of phosphate has been purified by ammonium sulphate precipitation and trypsin digestion. Its pH optimum is 9.5 and its properties are similar to the properties of alkaline phosphatase. Highly purified alkaline phosphatase from kidney and intestine prepared by three different methods also catalyzed this reaction. Complete conversion of TPN to DPN can be carried out with excess of enzyme which offers a method for the estimation of the individual nucleotides when they occur in mixtures in a single experiment.

Qualitative differences between erythrocytes from normal and tuberculous individuals. CURTIS SANDAGE AND YAYE FURUTANI (introduced by C. C. WANG). *Research Div. of Hines Veterans Hospital and Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

In a study of certain blood changes occurring in pulmonary tuberculosis in humans, the possible influence of qualitative differences in erythrocytes on the sedimentation rate has been investigated. 'Artificial' sedimentation rates were determined by suspending standardized, thoroughly washed red cells from various patients and normal persons in plasma from persons with high sedimentation rates. In general, there was an inverse correlation between the sedimentation rate of a given blood sample and the rate of settling of the same cells when washed and resuspended in another plasma. In such cases, normal cells showed very high sedimentation rates, whereas cells from advanced cases of tuberculosis settled very slowly. The separate effects of plasma on the sedimentation rate roughly paralleled the plasma content of polysaccharide associated with a_2-globulin as determined spectrophotometrically. However, that the qualitative difference between normal and tuberculous erythrocytes may be an immunologic phenomenon is suggested by the fact that *1)* erythrocytes from normal and tuberculous individuals were found to differ metabolically, as determined by manometric methods, and *2)* they differ antigenically as determined by cross absorption serological tests.

A dual-channel, piezoelectric, cathode-ray oscillographic recorder of muscle responses. ALEXANDER SANDOW (with the technical assistance of ARTHUR J. KAHN). *Washington Square College of Arts and Sciences, New York Univ., New York City.*

A frog skeletal muscle is isometrically linked to the stylus of a piezoelectric (phonograph) pickup connected to an amplifier stage forming an electronic differentiating circuit. This circuit accurately differentiates pulses with components up to 500–600 cycles/second; hence its output precisely gives the tension/time derivative of the contraction and relaxation periods of a twitch or tetanus. The latency relaxation (fundamental about 300–400 cycles/second) is imperfectly differ-

entiated. Following the differentiating stage, the pulse is fed into two separate channels. Channel I amplifies the contraction and relaxation periods' derivative and registers it on a sweep of one beam of a Du Mont dual-beam oscillograph. Channel II isolates the latency change (by clipping the relatively large derivative signal), reintegrates it to compensate for its initial imperfect differentiation, and, after amplification, registers it on a sweep of the oscillograph's second beam. The two sweeps, each suitably timed, are simultaneously tripped by the stimulus shock and each includes an appropriate timing wave impressed on its particular muscle-response deflection. For tetani longer than one-half second, sweep I is tripped by the first shock, and records the contraction period, and sweep II is triggered by a delayed pulse and records the relaxation period. Any of the above mechanical variations is recordable on sweep I and the muscle's action potential recordable on sweep II. In all cases separate optical myography registers initial and peak tension. (Aided under contract NR113-300 with Office of Naval Research.)

Massive pulmonary edema of central nervous system origin: hemodynamic observations and the role of sympathetic pathways. STANLEY J. SARNOFF *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

The injection of thrombin and fibrinogen into the cisterna magna of rabbits is followed by massive pulmonary edema which is lethal in from 5–20 minutes; this edema is accompanied by a rise in left auricular pressure. More detailed hemodynamic studies in the dog revealed a prompt and striking rise of pressures in the femoral artery, left auricle, pulmonary artery, and systemic veins. Left auricular pressures rose to as high as 80 mm. Hg. Vagotomy did not lower the elevated left auricular pressure. Data will be presented which suggest that the elevated pulmonary vascular pressures result from *a*) a drastic increase in the peripheral vascular resistance and *b*) a shift of blood from the peripheral to the pulmonary circulation. The administration of a sympathetic blocking agent in doses sufficient to reduce moderately the peripheral resistance was followed by a prompt return to normal of pulmonary vascular pressures and a rise in cardiac output. The ventricular work-failure threshold and its clinical implications will be discussed.

Effect of banthine upon gastric secretion and peptic ulcer—an experimental study. LESTER R. SAUVAGE* AND HENRY N. HARKINS. *Dept. of Surgery, Univ. of Washington School of Medicine, Seattle.*

Our experiments indicate that banthine, atropine and vagotomy will each prevent ulceration of the gastric rumen of starved rats subjected to the Shay procedure of pyloric ligation of 24 hours' duration. Banthine or atropine administered i.m. in beeswax 10 hours preceding the histamine test will reduce the gastric free hydrochloric acid secretory response by about 90%. In most cases banthine will prevent the free hydrochloric acid secretory response of the stomach to sham feeding employing dogs with simple gastric fistulae and divided double barreled cervical esophagostomies. Atropine does not result in so marked a diminution of the cephalic phase of gastric secretion. Banthine has little effect upon the secretory response of Heidenhain pouches in dogs following feeding. Banthine affords a degree of protection in the Mann-Williamson dog against ulceration of the jejunum adjacent to the gastrojejunostomy. However, the survival time of the treated dogs is not significantly increased over that of the controls. Of 6 controls all died with ulcer perforation or hemorrhage an average of 70 days after operation. Of 13 treated dogs one is still alive and in excellent condition while 12 are dead. Of these latter, 8 died without ulcer and 4 died with ulcer. Those dead without ulcer or still alive have survived postoperatively an average of 77 days. Those dying with ulcer did so an average of 59.5 days after surgery.

Inhibition of ovulation in the rabbit by banthine (β-diethylaminoethyl xanthene-9-carboxylate methobromide). CHARLES H. SAWYER, J. E. MARKEE AND JOHN W. EVERETT.* *Dept. of Anatomy, Duke Univ., Durham, N. C.*

Ovulation in the rabbit follows copulation-initiated neurogenic stimulation of the adenohypophysis. Earlier work from this laboratory has shown that the neurogenic stimulus involves an adrenergic mediator. Later results have indicated that a cholinergic mediator acts prior to, and may be responsible for, the release of the adrenergic mediator. The present work gives further indirect evidence that the stimulus contains a rapidly acting cholinergic component: rapid post-coital injections of banthine, a new potent anticholinergic agent, prevent ovulation in a significant number of animals. A series of 28/30 mature female rabbits ovulated in response to single matings. When copulation was followed within 30 seconds by intravenous injection of banthine (5 mg/kg.) ovulation failed in 5/10 cases. If the banthine was withheld until one minute post coitum, 8 of the same 10 rabbits, including 3 of the 5 earlier failures, ovulated. The other 2 animals ovulated when banthine was injected 5 minutes after mating. The blocking dose of banthine was found to develop protection against twice-lethal dosages of acetylcholine within a few seconds. The results

parallel those of our earlier work with atropine and are interpreted as confirmation of the existence of a rapid cholinergic component in the neurogenic stimulus for the release of ovulating hormone in the rabbit.

ACTH release in response to stress is partially prevented by an adrenergic blocking agent (SKF-501). CHARLES H. SAWYER AND GEORGE R. PARKERSON, JR.* *Dept. of Anatomy, Duke Univ. School of Medicine, Durham, N. C.*

Injected epinephrine stimulates ACTH release but there has been considerable disagreement as to whether an adrenergic mechanism is involved in the natural sequence by which stress activates the adenohypophysis. A barrier to the acceptance of the latter concept has been the general inability of adrenergic blocking agents to prevent stress-induced ACTH release. The present work is a study of the effects of a new, highly potent adrenergic blocking agent, SKF-501, on the eosinopenic response to epinephrine and cold stress in rats. In a dosage (10 mg/kg.) which was recently found effective in blocking the neurogenic stimulus for LH release in rats, SKF-501 itself acts as an alarming stimulus, inducing a marked eosinopenia at 4 hours. By 24 hours after injection the eosinophil count is back to normal but adrenergic blocking capacity is still high. At this time the usual effects of epinephrine (70% eosinophil loss) and cold stress (70% eosinophil loss) on ACTH release are partially blocked in the SKF-501-treated animals (only 25% and 50% eosinophil losses, respectively). The results are interpreted as support for the hypothesis that an intrinsic adrenergic mediator is utilized in the mechanism by which stress stimulates the release of pituitary ACTH.

Regional differences in CO_2 elimination through the skin. K. E. SCHAEFER (introduced by E. S. MENDELSON). *U. S. Naval Med. Research Lab., New London, Conn.*

In the experiments in collaboration with K. Scheer an infrared gas analyzer (absorption type after Luft) was used to determine the eliminations of CO_2 through the skin at 12 areas on 4 healthy subjects in a climate-chamber at 29°C. and 40% humidity. Remarkable uniformity was found for CO_2 elimination in corresponding skin regions from subject to subject. Cutaneous CO_2 elimination is approximately equal in the areas of the trunk, legs and arms. Considerably higher values were obtained from the skin of the face, plantar and palmar surfaces. Insignificant differences between right and left sides and between dorsal and ventral sides were noted. There appears to be a correspondence between cutaneous CO_2 elimination and cutaneous blood flow as found by Hertzman *et al.*

Preparation and physical studies of crystalline bovine albumin-fluorescein conjugates. RICHARD W. SCHAYER* AND ALFRED A. SCHILLER. *Rheumatic Fever Research Inst., Northwestern Univ. Med. School and Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Synthesis of fluorescein isocyanate and its conjugation with protein were effected by modifications of the methods of Coons *et al.* (*J. Immunol.* 45: 159, 1942). Fluorescein isocyanate added to a 2% solution of crystalline bovine albumin produced a yellow conjugate. After filtration and prolonged dialysis against saline and then water, the preparation was lyophilized producing a fluffy, orange-yellow, water soluble, fluorescent substance. About 80% yields were obtained. Preliminary physical studies indicate that the fluorescein-protein conjugate is not significantly different in physical properties from the native bovine albumin. The intrinsic viscosity, a measure of the shape of the molecule, was identical for the conjugate and the native protein. Sedimentation velocity measurements gave patterns showing a slight asymmetry suggesting that 5–10% of the material corresponds to large molecules. This may be due to the attachment of several fluorescein residues to a small percentage of the protein molecules. Electrophoretic studies indicate that the isoelectric point of the conjugate increased by 0.13 pH units suggesting that an average of 1.6 molecules of fluorescein had been attached to each molecule of protein. Fluorophotometry yielded similar results. Electrophoretic spreading experiments demonstrated only a slight increase in heterogeneity of the conjugate, supporting other evidence that probably one or two fluorescein molecules are attached to each protein molecule producing an average of 1.6. Spectral absorption curves of the conjugates revealed a peak at λ 490 mμ, identical with those of a Na fluorescein solution.

Parallelism of blood flow in renal cortex and medulla. ALLEN M. SCHER (introduced by LOREN D. CARLSON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn., and Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Recent studies have indicated a possible functional bypass of blood in the kidney. It has been maintained that the renal blood flow can pass almost entirely through a cortical or through a juxtamedullary-medullary route. Vascular channels have been described which might be the anatomical basis of such a shunt. Previous studies have not, however, employed direct or dynamic methods of studying flow in these two areas. Dye or radiopaque materials have been injected, and the presence or absence of the foreign sub-

stance interpreted as indicating flow changes. In the present experiments the thermoelectric blood flow recorder, first employed physiologically by Gibbs, was modified to record flow at a point in the kidney cortex and at another point in the medulla. The principle of the recorder is simple. The two junctions of a thermocouple are inserted in tissues of a living animal, and one junction is heated slightly above organ temperature. The temperature of the heated junction will be influenced by the flow near it. Increased flow will cool the heated junction, and with decreased flow the temperature of the heated junction will rise. Because both junctions are in the same tissue, the effect of blood temperature change is eliminated. Experiments have been done on dogs, cats and rabbits with this recorder. Since any nervous reflexes involving the kidney are considered to be mediated by the renal nerves or by one or another neurohormones, the renal nerves were stimulated at various frequencies, or l-epinephrine, l-arterenol and acetylcholine were administered. Most of these procedures caused a decrease in blood flow to the kidney although low frequency stimulation may increase flow and acetylcholine at times has increased flow. In all experiments, the changes in blood flow in the cortex and medulla of the kidney were parallel. Flows in these areas *never changed in opposite directions* as they would were there a 'shunt,' nor has flow in one area been passive while the other changed.

Fluorescein-conjugated plasma protein as an indicator of vascular permeability. ALFRED A. SCHILLER AND RICHARD W. SCHAYER.* *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, and Rheumatic Fever Research Inst., Northwestern Univ. Med. School, Chicago.*

Fluorescein has been conjugated to plasma protein by modification of the methods of Coons *et al.* (*J. Immunol.* 45: 159, 1942), providing an optically labelled green fluorescent colloid whose suitability for vascular permeability studies is indicated by the following evidence. *1*) Its rate of disappearance from the circulation indicates a 'half-life' of 1–2 days. *2*) When injected intradermally, its presence in the skin is detectable by its green fluorescence for 60 days; confirmed by biopsy. *3*) The depilated abdomens of rats given the conjugate i.v. were examined under u.v. 24 hours later, and the skin was without a trace of green fluorescence. Small areas of the skin were burned. Within 15 minutes to 1 hour the burned sites appeared green fluorescent, the intensities roughly paralleling the severity of the burns. *4*) Fluorescence microscopy of the tissue of rats revealed that the conjugate remained within the vascular tree. Green fluorescence, tentatively indicative of conjugate entry, was seen in proximal tubule cells of kidney, tissue granulocytes, Kupffer cells of liver, and questionably in hepatic cells. The procedure of conjugation does not confer antigenicity to the conjugates. On the basis of these preliminary studies it is believed that the protein conjugate is treated in the same manner as native plasma protein *in vivo*. Its optical label permits its qualitative and quantitative identification in tissues and body fluids by fluorometric techniques which can be adapted to specific problems of vascular permeability.

Experimental validity test of electrocardiographic dipole hypothesis and central terminal theory. OTTO H. SCHMITT, RAPHAEL B. LEVINE* AND ERNST SIMONSON. *Univ. of Minnesota, Minneapolis.*

Electrocardiographers agree that central terminal theory and the dipole hypothesis are very convenient concepts, but much controversy has arisen over the degree of their validity. To provide semi-quantitative criteria by which this situation can be clarified, a bridge balancing technique has been devised in which an exact null would be obtained for the potentials from any pair of points anatomically opposite each other along a line through the heart center if both concepts applied exactly. The perfection of balance is then used to assess the validity of theory. As a criterion of excellence, the ratio of the unbalanceable residual to the total of the contributing potentials is used and defines a cancellation coefficient. Experimentally thus far, a modified Wilson terminal has been used and pairs of balance points obtained in widely different directions through the chest for each subject. The subject is then placed in a device which locates spatially and records photographically the several radii. Although good cancellations are usually found, several of the connecting lines may fail to pass accurately through a center in the heart. Viewed frontally, the lines cross more nearly in a common point than when viewed from other directions. From this evidence we conclude tentatively that the dipole hypothesis is a usable concept, especially for the frontal plane, in spite of the heart's large volume and the anisotropy of the body. It seems clear also that a central terminal must include at least 3 potential components to be valid three-dimensionally or with chest electrodes. (Aided by a U. S. Public Health Service grant.)

Further observations on local responses of single medullated fibers in frog's phalangeal nerve. GORDON M. SCHOEPFLE AND JOSEPH ERLANGER. *Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

The method is the same as that previously

employed (SCHOEPFLE AND ERLANGER. *Am. J. Physiol.* 163: 748, 1950). The additional significant results are: *1*) The monophasic hump can attain $\frac{1}{10}$ the height of the spike, but with 'threshold' rectangular current stimulation the height can range down almost to invisibility, due to a 10% (ad max.) spontaneous variation in excitability. *2*) The hump appears to have a latency which is of the same order of magnitude as the discontinuity in excitability curves previously published (ERLANGER AND BLAIR. *Am. J. Physiol.* 99: 129, 1931), a discontinuity which, it now appears, is evident only when the rectangular stimulating current strength exceeded 80% of threshold, all of which is indicative of the development of a certain amount of refractoriness with the hump. *3*) Diphasic repetitive humps, seen occasionally, are indicative of a certain amount of conduction. The duration of the humps is slightly greater than that of monophasic spikes. *4*) When repetitive, the humps are not confluent as are Lorente's E_1 oscillations. *5*) Stopping the rectangular stimulating current during a hump does not stop its completion. The evidence indicates that the hump is the result of a partial break-down of the membrane. When a local, nonlinear catelectrotonic potential attains threshold through any time course it provokes instability and initiates either a local or propagated response. (Aided by grants from the Ella Sachs Plotz Fndn. and the U. S. Public Health Service.)

Nitrogen tension in the swimbladder of marine fishes in relation to the depth. P. F. SCHOLANDER. *Dept. of Biol. Chemistry, Harvard Med. School, Boston, Mass.*

Analysis of the gases of swimbladders from a variety of marine fishes caught at different depths have been made. On the reasonable assumption that nitrogen cannot be chemically secreted we would presume that the nitrogen tension in the swimbladder at a given depth, would in time reach equilibrium with the nitrogen tension in the surrounding seawater. As the latter at all depths is near 0.8 atm. it follows that the nitrogen percentage in the swimbladder should be approximately 80 divided by the total pressure in atmospheres. This proved essentially correct for most species. There is a general tendency, however, of a slightly elevated nitrogen tension the deeper one gets, at 20 atm. it may be more than one atmosphere above that of the seawater. In some species the correlation fails completely and the nitrogen percentage at all depths may be as high as in surface fishes, i.e. about 80%. By measuring in a pressure tank the depths at which these fishes just float it was always found to be near the surface, and they had therefore not secreted oxygen while in deep water. In most cases these fishes were known to be vertical migrators. It is hence believed that a greatly elevated nitrogen percentage in the swimbladder as compared with the depth is indicative of vertical migration. The reason for the tendency of all fishes to get some elevation of the nitrogen tension in the swimbladder with increasing depth is so far unexplained.

Pyridoxal phosphate as accelerator for kidney DOPA decarboxylase and its inactivation by DOPA and related substances. HERMANN F. SCHOTT (introduced by WILLIAM G. CLARK). *Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles.*

Guinea pig kidney enzyme prepared according to the method of Schales and Schales quantitatively decarboxylates small amounts of DOPA; larger amounts however are only partially decarboxylated and if the DOPA concentration is further increased the absolute rate is less than for small amounts. This inhibition does not occur if added pyridoxal phosphate is present. If the pyridoxal phosphate however is first incubated with a small amount of DOPA its activating property is completely destroyed. Phenylalanine, tyrosine, 3-methyltyrosine, 3-fluorotyrosine and catechol do not similarly inactivate pyridoxal phosphate, while 3-4 di-hydroxyphenylserine, and 3-hydroxytyramine do. Glutatihone, phenylalanine and certain other amino acids prevent inactivation. Further studies on the structure-activity relationships of this inhibition will be discussed. The absorption spectrum and other properties of pyridoxal phosphate and its reaction product were compared with those of pyridoxal and pyridoxamine and the kinetic effect of concentration and pH changes were followed.

Titration of extracts and hormones of adrenal cortex by the method of unstained cell counts and by a color test. ROBERT SCHREK (introduced by JULES H. LAST). *Research Service, Veterans Administration Hospital, Hines, Ill.*

Cell suspensions from the rabbit thymus were incubated with extracts and hormones of the adrenal cortex for 24 hours at 37° C. To determine the number of viable cells, a solution of safranine was added to the treated and untreated suspensions and the unstained, i.e. viable, cells were counted in a hemocytometer. A unit of extract or hormone was defined as the amount of reagent which, on incubation with the cell suspension, resulted in a viable cell count of 50% of the control incubated suspension without reagent. Another method of titration depended on the capacity of viable cells in a suspension to reduce the redox indicator, 2,6 dichlorophenol indophenol. The suspension was incubated with the reagent for 24 hours and then the capacity of the suspension

to reduce the indicator was tested. A failure of the indicator to be reduced as much as in the control suspension indicated that the reagent had a toxic effect on the cells. As a standard, a mixture of equal parts of fresh and inactivated (56° 1 hour) suspension was incubated. The treated suspension that had the same capacity to reduce the dye as the standard was said to have 1 unit of reagent. By the method of unstained cell counts, one unit was equivalent to 0.03 gamma of compound F or 0.00,03 glycogen deposition units. Several pure hormones and various extracts of the adrenal cortex are now being titrated by the present methods.

Depressor effects of certain sulfhydryl compounds. HENRY A. SCHROEDER AND EDNA M. MENHARD.* *Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.*

The intravenous injection of cysteine into anesthetized renal hypertensive rats was followed in 5 minutes by a fall of blood pressure lasting 1 or 2 hours, which did not occur in normotensive rats. This response did not follow the injection of cystine, methionine, 1-methyl, 2-mercaptoimidazole, 1,2 mercaptobenzoxole, thiosalicylic acid; 2-mercaptoethanol sometimes caused it, but sodium thioglycollate constantly did. Responses to these compounds by normotensive rats were inconsistent. In unanesthetized hypertensive rats intramuscular 2,3-dimercaptopropanol caused consistent lowering of systolic pressure lasting 2 or 3 hours but not 4 hours. In unanesthetized renal hypertensive dogs mean pressure became considerably lower for 2–4 hours after injection of cysteine, sodium thioglycollate and 2,3-dimercaptopropanol; the same response was observed in 3 hypertensive patients. Repeated injections resulted in no persistent effects. Shock was not produced. Cysteine did not modify the pressor response of rats to nor-epinephrine. It is believed that sulfhydryl compounds of a certain configuration may sometimes act as transient depressor substances and that this reaction may be confined to some hypertensive states.

Hearing loss after experimental injury to apex of the cochlea. H. F. SCHUKNECHT* AND W. D. NEFF. *Div. of Otolaryngology and Lab. of Physiological Psychology, Univ. of Chicago, Chicago, Ill.*

There is abundant evidence that high tones stimulate receptors in the basal end of the cochlea and that damage to the receptors or neural elements in this region results in high tone deafness. The picture with regard to low tones is much less clear. Apparently frequencies in the lower range (1000 cps and below) produce a widespread response within the cochlea with the region of maximal effect being near the apex. An inner ear type deafness affecting only the low frequencies is rare in clinical experience; in the early stages of Meniere's disease a loss of hearing involving the low tones over a range of several octaves is sometimes seen. The present experiment was undertaken to obtain further information as to the degree of localization of low tones in the cochlea. An operative method was developed by which a small circumscribed lesion could be made in the apical turn of the cochlea. Audiograms of experimental animals (cats) were determined by avoidance conditioning procedure before and after making the cochlear lesion. After post-operative testing, cochleas were sectioned and graphic reconstructions made. The locus and extent of injury were correlated with hearing losses. Lesions limited to a small region in the apical turn have been produced successfully. In some cases impairment of hearing for low tones with approximately normal hearing for high tones has occurred. The hearing loss is not as abrupt nor as severe as that reported for similar lesions in the basal turn.

Effect of hypophysectomy on electrolyte and water balance in dog. I. L. SCHWARTZ,* D. P. EARLE, JR., R. C. DE BODO, S. J. FARBER,* M. KURTZ* AND J. GREENBERG.* *Depts. of Pharmacology, Medicine and Physiology, New York Univ. College of Medicine, New York City.*

Thirteen hypophysectomized dogs were studied. To date serial sections of the pituitary areas of 5 dogs have been examined. No adenohypophyseal cells were seen in any. The adrenals showed great weight reductions and atrophic changes in all 3 cortical layers (N. LANE. *Federation Proc.* this issue). Marked insulin hypersensitivity and inability to withstand fasting indicated disturbance of carbohydrate and protein metabolism. Glomerular filtration rate and renal plasma flow were considerably reduced. Orally administered water load (40 ml/kg.) was well absorbed but its excretion was retarded (4–45%, average 25% within 3 hours as compared to 100% in normals). However, the animals withstood low sodium diets (1 to 2 mEq/day) for 3 weeks without ill effect and without change in serum sodium concentration. Large doses of potassium (0.3 to 0.98 mEq/min. for 2.5 hours) were administered intravenously to 4 dogs that had been given oral potassium for 3 to 4 days beforehand. During the potassium infusion, potassium excretion increased promptly approaching or exceeding the filtered load. The plasma potassium did not reach toxic levels in spite of the low filtration rates. Three to 15 weeks following hypophysectomy, T-1824, inulin, deuterium oxide, and Na^{24} spaces were within normal limits. It is concluded that hy-

pophysectomized dogs exhibiting atrophic changes of all adrenal cortical layers differ from adrenalectomized animals in that their water and electrolyte distribution and their responses to potassium load and sodium restriction are normal.

Peptidase activity of surviving rat diaphragm. THEODORE B. SCHWARTZ,* FRANK L. ENGEL AND CATHERINE C. TOWBIN.* *Dept. of Medicine, Duke Univ., Durham, N. C.*

A technique which permits a quantitative estimate of the rate of hydrolysis of glyclygylcine (GG) by the surviving rat diaphragm has been devised. A freshly removed hemidiaphragm is suspended in an artificial medium (CHRISTENSEN, H. N. AND J. STREICHER. *Arch. Biochem.* 23: 96, 1949) in an atmosphere of 95% O_2 and 5% CO_2. After washing 4 times in fresh medium, the hemidiaphragm is transferred, under the same conditions, to a similar medium containing 10.7 mM/l. of GG. After a specified time, the hemidiaphragm and medium are removed and free glycine concentrations are determined (ALEXANDER, B. *et al. J. Biol. Chem.* 160: 51, 1945) on picrate supernates. The GG hydrolyzed, expressed as μM/gm. of diaphragm, is calculated as: $\frac{1}{2}$ $[(GD_1 + GM_1) - (GD_0 + GM_0)]$, where GD_1 represents the glycine concentration (μM/gm. diaphragm) in the hemidiaphragm exposed to GG; GM_1 the glycine concentration (μM/ml.) of the GG-containing medium; and GD_0 and GM_0, the glycine concentrations of control hemidiaphragm and medium, respectively. Various control experiments have been interpreted to indicate that the major site of GG-splitting activity is *within* the diaphragm. Since it has been found that the amount of GG hydrolyzed is proportional to the time of exposure to the medium containing GG, dipeptidase (GGase) activity may be defined as GG split/gm. of diaphragm/minute. In the presence of 0.001M Co^{++}, GGase activity of surviving diaphragm, like that of muscle extracts (SMITH, E. L. *J. Biol. Chem.* 173: 571, 1948), is enhanced. A quantitative comparison indicated that cobalt-activated GGase activity of intact diaphragm is only about $\frac{1}{50}$ that of an aqueous extract of diaphragm.

Response of Pacinian corpuscle to oscillatory stimulation. DONALD SCOTT, JR. *Dept. of Anatomy, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

A preparation has previously been described in which a single Pacinian corpuscle in the mesentery of the cat can be dissected free of connective tissue while the neural and vascular connections are maintained intact. Such a preparation can be stimulated by oscillating pressure of extremely low energy (approaching the level required to stimulate the human ear) and the electrical activity recorded by fine cotton wisp electrodes. The threshold curve shows a sharp dip at a frequency corresponding to the fundamental period of the corpuscle. Similar dips are also found at frequencies which are simple sub-harmonics of the primary frequency but the dips become progressively shallower as frequency decreases. This would be expected if the rate of change of deformation were the effective stimulus for the corpuscle. If the response of two or three corpuscles is studied simultaneously the same type of threshold curve appears but the dips appear in duplicate or triplicate corresponding to the number of corpuscles. Such results strongly suggest that the sharp dips in the threshold curves are truly physiological in origin and not the result of resonance of some part of the apparatus. This provides further evidence in support of the belief that the activity of the corpuscle is largely affected by continuous naturally occurring oscillations of its excitability.

Electrocardiographic effects of vagal stimulation. J. C. SCOTT AND E. A. REED. *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

Electrocardiograms (lead II) were taken on 40 dogs under Nembutal, Pentothal or chlorotone anesthesia during carotid sinus stimulation, local cooling of the SA node or a combination of these procedures. In most experiments the exposed sinuses were stimulated by manual compression. In many instances the procedure was repeated following unilateral and bilateral cervical vagotomy or thoracic sympathectomy. Particular attention was given to the R - R and P - R intervals and the amplitude of the P wave. In general, stimulation produced an increase in the duration of R - R and P - R and a decreased P amplitude. The average R - R increased proportionally with the P - R during stimulation. This relationship is influenced by the anesthetic employed. Maximum response of all three factors occurs in the first or second cycle and is much diminished by the tenth cycle. Local cooling of the SA node prolongs the R - R and decreases P amplitude more than vagal stimulation and frequently shortens the P - R interval. Sufficient cooling induces nodal rhythm. Stimulation during the application of cold to the SA node produces a greater R - R value in both sinus and non sinus rhythms and may also increase the P - R and P responses during sinus rhythm. Unilateral sympathectomy (stellate to T IV or V) elevates resting P values but has no marked effect upon response to stimulation or to cold. Bilateral sympathectomy depresses the resting P value but has only slight effects upon response to stimulation. Bilateral vagotomy with or without sympathectomy abolishes the response

to stimulation without effecting the reactions to local cooling.

Effect of ox bile on fat absorption in bile fistula dogs. G. W. SEARLE* AND J. H. ANNEGERS. *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Since previous studies showed that whole dog bile must be returned to the duodenum nearly continuously to effect normal fat absorption in bile fistula dogs, substitution of solutions of Extract of Ox Bile, U.S.P., for whole dog bile has been tested under these conditions. Daily doses of 0, 7, 10, and 13 gm. of ox bile, and mixed diets containing 36 and 51 gm/day of fat were used. Feces from the last 5 days of 8-day periods were analyzed for total fat (FOWWEATHER AND ANDERSON. *Biochem. J.* 40: 350, 1946). Data from 32 tests on 19 dogs showed that for a given dose of ox bile a constant percentage of dietary fat was absorbed. Thus, when no bile was returned, fat absorption was 52%, in agreement with previous work, and with 7, 10, and 13 gm/day of ox bile fat absorption averaged 75%, 86%, and 93%, respectively, of either dietary fat level.

Attempts to demonstrate alloxan in human urine. DAVID SELIGSON,* HARRIET SELIGSON,* BERNARD SHAPIRO,* RONALD G. PALEY,* TANIA RIABOFF* AND F. D. W. LUKENS. *George S. Cox Med. Research Inst., Univ. of Pennsylvania, Philadelphia.*

Suggestions have been made in the literature that the diabetogenic compound, alloxan, might be concerned in the pathogenesis of human diabetes. Our studies showed that in plasma alloxan was rapidly and almost completely converted to alloxanic acid under the conditions employed. The reaction proceeds spontaneously in neutral or alkaline solutions. Alloxan injected into rats is excreted partly as alloxanic acid. For these reasons a search was made for alloxanic acid rather than alloxan which disappears rapidly. When either alloxan or alloxanic acid is hydrolyzed in alkali, the salt of oxomalonic acid is formed. The 2, 4-dinitrophenylhydrazone of oxomalonic acid is easily isolated by partition chromatography on paper or silica by methods to be reported. Urine samples from 15 consecutive persons, normal and diabetic, were partially purified and hydrolyzed in alkali. The keto-acids were converted to the 2, 4-dinitrophenylhydrazones and this group of compounds was fractionated. Oxomalonic acid was found in all urines in varying amounts. Because oxomalonic acid is the carbon moiety of alloxan and of alloxanic acid (the other part being urea), it is possible that normal and diabetic persons excrete alloxanic acid or a closely related compound. Our experience suggests that alloxan might well have been the precursor of the oxomalonic acid found in these subjects.

Effect of pulse pressure and mean arterial pressure modification on renal function. EWALD E. SELKURT. *Dept. of Physiology, Western Reserve School of Medicine, Cleveland, Ohio.*

In dogs anesthetized with pentobarbital arterial pulse pressure was varied with inclusion of a damping chamber in an external circuit perfusing the kidney from the carotid artery. Mean pressure was varied by means of a clamp in the circuit. Pressures were measured optically. When the pulse was damped at normal pressure levels, no effect was noted on the clearance of creatinine, PAH, sodium excretion, and urine volume. In another group of animals, the mean pressure was first reduced to *ca* 90 mm. Hg, following which the pulse was eliminated. Again no effect was noted on the clearances. However, the reduction in mean pressure usually had a marked effect on sodium excretion and urine volume. They were decreased despite the fact that PAH clearance remained constant, and creatinine decreased little. This suggested that an underlying mechanism distinct from alteration of sodium load was operative. It is suggested that the reduction in perfusion pressure slowed the rate of tubular urine movement, aiding in more complete reabsorption of sodium and water.

Potassium movement and nerve activity. ABRAHAM M. SHANES. *Natl. Insts. of Health, Bethesda, Md.*

Libinia emarginata. Polarization changes of the leg nerves have been observed during and following repetitive stimulation. The depolarization resulting from 2 supramaximal shocks/sec. attains a maximum of 2.8 mv. in about 5 minutes and is doubled by 0.05 mg.% veratrine hydrochloride; repolarization during recovery is appreciably slower but greater and thus gives rise to a positive after-potential. Potassium shifts were studied under similar conditions with a unit permitting stimulation, observation of spikes, and recirculation of media of less than 1 ml. Analyses of these media demonstrated a potassium release of 3×10^{-3} μM/impulse, gm. wet weight which is increased 65% by 0.02 mg.% veratrine; during recovery reabsorption continued for over an hour and exceeded the initial loss. The direction and magnitude of potassium movement therefore support the hypothesis that the after-potentials reflect changes in the potassium concentration at the fiber surface. *Loligo pealii.* The inexcitability of giant axons, resulting in nitrogen gas within 0.5 hour, can be washed away repeatedly within seconds with oxygen-free solution, but disappears only after minutes in oxygen gas; this emphasizes

the importance of surface potassium in spike production and indicates that aerobic metabolism contributes indirectly. *Rana catesbiana*. Stimulation of intact sciatic nerves for 1 hour at 100 shocks/sec. caused a potassium release of 1.3×10^{-6} μM/impulse, gm. wet weight. Removal of the epineurium increased the loss 15 fold—a value also obtained with stimulation at 20 shocks/sec. for 0.5 hour; 0.1 mg.% veratrine more than doubled potassium escape during activity.

Effect of autonomic blocking agents on gastric secretion in the rat. HARRY SHAY, MARGOT GRUENSTEIN* AND WILLIAM JAMISON.* *Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.*

Using the rat preparation developed in our laboratory (*Gastroenterology* 5: 43, 1945) we studied the effects of various autonomic blocking agents on gastric secretion. In two groups of properly starved rats the pylorus was ligated, the stomach lavaged with saline and emptied completely. In one group, the drug dissolved in normal saline was injected subcutaneously. Similar amounts of normal saline alone were injected in the controls. In the other group of rats, the drugs were administered intraduodenally by injection at the time of pyloric ligation and its controls were given the same amounts of normal saline intraduodenally. Four hours after pyloric ligation and administration of the drug, the animals were sacrificed and the gastric contents were collected quantitatively. These were measured and analyzed for free and total acidity, pH, chloride, pepsin concentration and activity when enough material was available. In some cases, we had to pool specimens to permit all of these determinations. Volume of secretion per hour per 100 gm. body weight was calculated. Drugs studied were Atropine, Banthine, Dibutoline, Artane, Hexamethonium iodide, Dibenamine, and Tetraethylammonium. The same doses by weight per body weight of all drugs were used. Atropine and Banthine produced the most striking effects on gastric secretion. These were essentially the same with the two drugs. The detailed results with the various drugs used will be presented.

Lack of an oxygen effect in the disturbance by x-rays of selective potassium accumulation in human erythrocytes in vitro. C. W. SHEPPARD AND MARIAN STEWART.* *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

When heparinized human whole blood is exposed to x-rays (30,000 r) and subsequently equilibrated *in vitro* under quasi-physiological conditions, potassium progressively leaves the cells and sodium penetrates in essentially equimolal amounts (SHEPPARD. *Biol. Bull.* 93, 310). Experiments were performed in which blood was equilibrated with an atmosphere containing 5% CO_2 and 95% He or O_2. The completeness of the reduction or oxygenation of the blood was checked by spectrophotometry of the hemoglobin. After x-ray exposure the potassium leakage rate was determined by serial flame spectrophotometry of plasma samples. No difference could be detected between the leakage rates in samples irradiated under He or under O_2. This finding is in distinct contrast to the widespread depression of x-ray effects in many other tissues when irradiated under reduced oxygen tension.

Effect of x-radiation on fermentation of glucose by low and high nitrogen yeast. F. G. SHERMAN. *Biology Dept., Brown Univ., Providence, R. I.*

Yeast cells differing in total nitrogen content were produced by growing yeast (belonging to either of two strains of *S. cerevisiae*) in a synthetic medium containing different known amounts of $(NH_4)_2SO_4$ as the only nitrogen source. Cells washed and adjusted to standard density with phosphate buffer (pH 4.5) were irradiated in pyrex test tubes with a therapy tube, employing the following irradiation factors: 100 kv., 10 ma., dose rate 1410 r/minute, no additional filtration. Total doses of 10,000–60,000 r were applied. After irradiation, cell suspensions were incubated at 30° C. for 4 hours. Aliquots were taken for determination of total nitrogen and for manometric estimation of anaerobic CO_2 production. Non-irradiated cell suspensions, otherwise treated like the experimentals, served as controls. The anaerobic CO_2 production of low nitrogen yeast grown in medium containing 0.01 gm. $(NH_4)_2SO_4$/l. was inhibited 50–60% by 30,000 r. The anaerobic CO_2 production of yeast grown in medium containing 1.0 gm. $(NH_4)_2SO_4$/l. and irradiated under the same conditions was inhibited only 14–25%. Low nitrogen yeast cells were more sensitive to the effects of irradiation than high nitrogen yeast cells throughout the dose range employed. The fermentation of glucose by these two yeast strains grown in a yeast extract-peptone medium was not inhibited by even much larger doses.

Excitability cycle of the intact dog heart. R. S. SIKAND,* H. M. GELLER,* H. LEVINE* AND L. H. NAHUM. *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The excitability cycle of the intact dog heart was determined by a method which enables the relationships between the various phases of excitability and electrical events to be determined, and which avoids the errors that accrue from an unphysiological preparation. Dogs were anesthetized with sodium pentobarbital (Nembutal) and maintained in a steady state of anesthesia. A unipolar lead from the left precordium was fed

into an R.-C. coupled amplifier whose output triggered a variable delayer circuit. The amplitude discrimination portion of this circuit determined which part of the QRS complex would activate the variable delay. The output of the delayer was led to a square wave generator, stimuli from which were employed for testing the response of the myocardium. Concentric electrodes were introduced through the intact chest wall for stimulation. Testing stimuli were applied once every 2 minutes with an interposed mercury key. A cathode-ray oscillograph was used for monitoring throughout the experiment. The representative excitability curve at the left ventricular apex demonstrates that the absolute refractory period ends soon after the peak of the T wave and the threshold period begins shortly before the end of T. At the base of the ventricle the absolute refractory period terminates shortly before the end of T and the threshold period begins soon after. The thresholds during the various phases of excitability are lower at the apex than at the base. These values remained constant for many hours, provided that rate, state of anesthesia, and site of the electrodes remained unchanged.

Sodium homeostasis in hypophysectomized dogs. D. H. Simmons, R. B. Harvey, T. Hoshiko and D. Ferguson (introduced by M. B. Visscher). *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

Five totally hypophysectomized dogs were subjected to gradually increasing rates of continuous intravenous infusion of 1.5% NaCl solution over a period of approximately one week. Sodium chloride intake by this means reached levels as high as 12.5 gm/kg. body weight/day. At all levels of NaCl intake there were no observable differences in this ability to adjust salt excretion rates from that of 3 normals. Four of the hypophysectomized dogs were also placed on a rice diet for one week. Following both types of preparation, the dogs were then subjected to osmotic diuresis with 1.85 molal mannitol solution (25% by weight) containing creatinine given at the rate of 0.44 cc/min/kg. body weight. Urine and serum creatinine and sodium determinations were made using the standard Evelyn colorimeter method and the Beckman flame photometer, respectively. The two preparatory procedures and the osmotic diuresis were repeated on three normal controls. The tubular rejection fraction of water at which the diuresis began to cause an increased tubular rejection fraction of sodium was used as a measure of the sodium reabsorptive capacity of the tubules. While the level of sodium chloride intake markedly affected the point at which excess sodium loss began for all animals, hypophysectomy had no observable effect. The ability of the totally hypophysectomized dogs to adjust to varying rates of sodium chloride intake and the similarity of their response to that of normal dogs during osmotic diuresis suggests that neither the anterior nor the posterior pituitary play indispensable roles in sodium homeostasis.

Interpretation of electrocardiographic mirror patterns. Ernst Simonson, Otto H. Schmitt, Raphael B. Levine* and Ancel Keys. *Dept. of Physiological Hygiene and Biophysics, Univ. of Minnesota, Minneapolis.*

Most electrocardiographers interpret precordial and unipolar limb leads as local patterns attributable to epicardial areas facing the electrodes, but Duchosal and Sulzer claim that any lead records a central vector. The technique described in the preceding paper (Schmitt *et al.*) was used to decide this question. The distribution of cardiac potentials over the chest was mapped in 11 normal subjects and electrocardiograms were taken from the cross-sections of 13 evenly spaced vertical lines and 6 horizontal lines corresponding to the intercostal spaces as well as from several additional points including the limbs amounting to a total of 90 leads. Under guidance from the electrocardiographic map the following patterns (and their mirror patterns) were selected for cancellation: rS, T−(qR, T+); rS, T+(qR, T−); RS, T+(QR, T−); qR or R, T+(QS, T−) and limb potentials. QRS as well as T deflections could be cancelled, though often not in the same location. Of a total of 102 QRS cancellations, the cancellation was excellent in 38 (cancellation-coefficient 0.0–0.07) good in 27 (0.08–0.11), fair in 16 (0.12-0.15), poor in 21 (>0.15). There was no significant difference in the completeness of cancellation between precordial and distant leads, or between various electrical heart positions, though the electrical heart position affects the anatomical pattern distribution. The results support Duchosal and Sulzer's hypothesis.

Water and electrolyte disturbances in acute renal insufficiency. Jonas H. Sirota, and Irving Kroop (introduced by Milton Mendlowitz). *Dept. of Medicine, Mount Sinai Hosp., New York City.*

Inulin spaces were determined in 4 patients, and water and electrolyte balances studied in 6 patients with acute renal insufficiency resulting from intoxication by carbon tetrachloride in 4 of the subjects and sulfonamides in two.

The inulin spaces were expanded 2.9, 3.8, 9.1, and 4.8 liters above the expected normal extracellular fluid volumes. The net fluid balances from the onset of oliguria to the time of inulin space determinations were +0.7, −1.0, +3.4 and +2.1 liters respectively. These time intervals were 4,

8, 10, and 8 days respectively. A minimal insensible fluid loss of 500 cc/day was assumed. The sodium intake was sufficient to account for the degree of extracellular expansion in 3 of the 4 subjects. The 4th subject had 3.0 liters of extracellular fluid in excess of that explainable by water and sodium intake. Hyponatremia (from 108 to 124 mEq/l.) was present during oliguria. With diuresis the plasma sodium concentration gradually returned to normal in spite of urinary loss of sodium far in excess of intake. It is suggested that hyponatremia during oliguria may result in part from dilution of the extracellular solutes by water fron the cellular phase, and in part from a shift of sodium into cells. Hyperkalemia (from 4.5 to 10.8 mEq/l.) was present during oliguria. Plasma potassium concentrations remained constant or increased during early diuresis. This may be explained on the basis of a contracting extracellular fluid volume in the face of only a slight increment of potassium excretion. These studies suggest that the oliguria of acute renal insufficiency is associated with cellular dehydration resulting from a shift of hypotonic fluid out of the cells.

Absorption and excretion studies of alkyl sugar derivatives. Floyd R. Skelton, Gordon A. Grant and John K. Souch. (introduced by Hans Selye). *Research and Biological Labs., Ayerst, McKenna and Harrison Limited, Montreal, Quebec, Canada.*

The absorption and urinary excretion characteristic of each of several alkyl derivaties of glucose and fructose were studied in the normal rat after a single oral and single intraperitoneal dose. Approximately the same percentage excretion (75–90%) of 3-methylglucose was found with either route of administration while 3-ethylglucose, 3 butylglucose and 3-propylglucose were almost quantitatively excreted following intraperitoneal injection but were only 25, 27 and 35% respectively excreted after oral feeding. Similarly, 3-methylfructose and 1-methylfructose were almost totally excreted in the urine after intraperitoneal administration but were respectively only 20 and 60% excreted when given orally. Balance studies in which 3-methylglucose, 3 ethylglucose, 3-methylfructose and 1-methylfructose were fed to rats as 20% of their diet confirmed the excretion values obtained with a single dose. All sugar derivatives except 3-methylglucose caused a marked diarrhea undoubtedly due to their slower absorption, a fact which also accounts for the lower urinary excretion found. A diuresis proportional to the sugar excretion was observed in all treated groups, being greatest in those rats receiving 3-methylglucose. In addition 3-methylglucose was fed to male and female rats as 20% of their diet for a period of 1 year. At the end of this time no adaptation to the sugar appeared to have developed since its absorption and excretion was the same as initially observed. Furthermore no gross or microscopic lesions were detected which could be ascribed to the sugar administration.

Effects of adrenergic blocking agents on thyroid hyperplasia produced by allyl thiourea in rats. Stanley C. Skoryna, D. R. Webster and D. J. Beauprie (introduced by F. C. MacIntosh). *Dept. of Experimental Surgery, McGill Univ., Montreal, Quebec, Canada.*

Different results have been reported by the various workers who have investigated histologic changes and thyroid function following complete or partial denervation of the thyroid gland. A study is being carried out of the effects of certain autonomic blocking agents on the hyperplastic process in the thyroid produced by the so-called goitrogenic (anti-thyroid) drugs. In the present experiment allyl thiourea was administered continuously to young rats of both sexes by adding it to standard diet: treatment was begun at 7–9 weeks; concentration in the food was 0.046% for the first 2 months, and 0.06% for the following period. Beginning 6 months after the start of the experiment, the rats received in addition Dibenamine (250 mg/kg/day) by intraoesophageal instillation. Animals were killed at intervals to determine the course of histologic changes. An increase in proliferative hyperplasia was observed when the blocking agent was administered along with the goitrogen. It is suggested that Dibenamine, in addition to its powerful adrenaline-blocking action, has some inhibitory effect on tonic vasoconstrictor impulses and so increases the blood flow to the hyperplastic gland. Oxygen consumption was measured in the same animals by the method of Farmer and Crampton: the results will be reported.

Hemodynamic changes resulting from insertion of a rotometer in the venous circulation of a dog. Edwin L. Smith and Russell A. Huggins. *Dept. of Physiology, Univ. of Texas Dental School, and Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

Dogs were anesthetized with morphine 10 mg/kg. subcutaneously followed by Na pentobarbital 10 mg/kg. intravenously. The chest was opened by a midline incision. A Hamilton manometer was used to measure arterial pressure from the aortic arch and water manometers were used for the venous pressure measurements. A large recording rotometer was inserted to measure total venous return. Chlorazol fast pink or heparin was used as the anticoagulant. After insertion of the rotometer it was observed that arterial pressure and venous return slowly declined to low levels with

death occurring in 2–4 hours. Massive transfusions gave only slight to moderate improvement in pressure and flow, any improvement was lost in 15–20 minutes after stopping the transfusion. Venous pressures peripheral to the rotometer increased a variable amount while right auricular pressure decreased slightly and arterial pressure dropped to between 30–35 mm. Hg. Duplication of the procedures without insertion of the rotometer did not cause the deterioration of the dogs. Analysis of the pressure loss due to the rotometer and necessary cannulae and tubing indicates that the dogs did not raise their venous pressure sufficiently to maintain a normal venous return through the rotometer.

Circulating blood cells following radiation in hibernating woodchuck. FALCONER SMITH AND MARIE M. GRENAN.* *Natl. Insts. of Health, Bethesda, Md.*

The rate of development of the lethal processes following radiation has been shown to bear a close relationship to the metabolic rate. Changes in the circulating blood cells provide a useful index of the rate at which radiation sequelae develop. Standard procedures were followed in studying 8 woodchucks irradiated while in hibernation and 10 animals which served as irradiated non-hibernating controls. The non-hibernating animals were given 550 r which was estimated to be near the LD_{100} and, in order to emphasize a possible protective effect of hibernation, the hibernators were given 650 r. Circulating leucocytes and erythrocytes undergo no significant quantitative change during hibernation in the irradiated woodchuck up to 42 days after irradiation. Immediately upon the termination of hibernation, with the return of the animals to room temperature, these blood components rapidly diminish in number until the time of death 4–10 days later. Differential counts revealed essentially no change until the end of hibernation in the irradiated woodchucks. Studies of the bone marrow of 2 hibernating woodchucks which were irradiated 3 weeks previously show evidence of severe damage, although throughout the hibernating period the circulating leucocytes were not reduced in number when compared with the hibernating non-irradiated controls.

Thermal fragility of rat erythrocytes following whole-body x-irradiation. FALCONER SMITH AND MARIE M. GRENAN.* *Natl. Insts. of Health, Bethesda, Md.*

Changes in total blood lipids have been reported to follow whole-body irradiation. We have tested the integrity of the erythrocyte membrane in rats by determining its thermal fragility. Measurements were made in a Klett photoelectric colorimeter following incubation for a maximum of 60 minutes at 53°C. The mean time for 50% hemolysis (H_{50}) was 44.4 ± 5.9 minutes for heart blood drawn from 18 healthy male rats (Marshall strain) averaging 185 gm. in weight. In one experiment 15 rats were given an x-ray dose of 620 r which regularly kills all of this strain in our laboratory. At each interval of 12, 20, and 40 hours after radiation heart blood was obtained from 5 rats. Mean H_{50} values for these blood samples were 41.3, 42.5, and 42.6 minutes, respectively. In another experiment 24 rats of the same strain and weight were given 1000 r. H_{50} values were determined on blood drawn from 6 animals at each of the following times after radiation: 4, 20, 36, 60 hours. The mean times for 50% hemolysis were 42.5, 43.8, 39.7 and 42.9 minutes, respectively. No significant alteration in thermal fragility of erythrocytes was found to occur up to 60 hours after lethal doses of x-rays in these experiments.

Dual role of thiamine in producing bronchoconstriction. JAY A. SMITH AND HERBERT SOHN (introduced by A. H. RYAN). *Dept. of Physiology and Pharmacology, Chicago Med. School, Chicago, Ill.*

Curare preparations have been shown to produce bronchoconstriction probably by liberating histamine; thiamine, in large doses, has curariform activity. It was desirable to know if thiamine could cause bronchoconstriction, particularly since effects typical of anaphylaxis, including death, have been reported from administering thiamine to human patients. Dogs were anesthetized with sodium pentobarbital (Nembutal); negative pressure artificial respiration was applied to the intrathoracic space; oxygen was supplied by intratracheal insufflation; intratracheal pressure was recorded as an index of bronchial constriction; ethyl alcohol, about 3 cc., was injected slowly into the cisterna magna to paralyze respiration and to reduce autonomic activity. Thiamine hydrochloride, 100 mg/kg., was injected intravenously. In some dogs, benedryl, 5 mg/kg., was injected intraperitoneally not less than 3 hours before injection of thiamine. It was found that all dogs in which thiamine caused a pronounced hypotension also showed bronchoconstriction, which, in most cases, was roughly proportional to the fall in blood pressure (and hence proportional to paralysis of sympathetic tone). However, in several dogs not receiving benedryl, the blood pressure was already low (as a result of the injection of alcohol); in some of these, thiamine produced very little further fall in blood pressure, but, nevertheless, a well defined bronchoconstriction. This was likely due to liberation of histamine, because in other dogs with low blood pressure which received benedryl, thiamine caused no further fall in blood pressure and no bronchocon-

striction. From these results it is concluded that bronchoconstriction produced by thiamine can be due to block of the sympathetc system and to liberation of histamine.

Effect of ACTH on distribution of plasma proteins of patients with pulmonary tuberculosis. LEONARD C. SMITH (introduced by C. C. WANG). *Research Div. of Hines Hospital, Hines, Ill.*

The changes which occur in the plasma proteins of tuberculous patients following the administration of ACTH were demonstrated by use of the Tiselius electrophoresis technique. Heparinized plasma samples from 12 tuberculous patients were studied before and at 2-week intervals during the time of ACTH administration. The data reveal an increase in the albumin fraction from an average value of 33% to approximately 42%. At the same time, the fibrinogen fraction decreased from an average value of approximately 14% to 10% while the gamma globulin component likewise decreased from an average value of 20% to about 13%. The values for the alpha 1, alpha 2, and beta globulins, although not distinctly abnormal from the beginning, appeared to be variable. The A/G ratios in most cases showed a definite increase. In general, the trend of the various fractions was toward the normal range with the exception of one patient who did not appear to respond to ACTH. It was also observed that reduction or cessation of ACTH brought about a reversion of the protein fractions to values similar to those noted in the control samples.

Influence of the adrenal on response of the rat mammary gland to ovarian steroids and relaxin. THOMAS C. SMITH (introduced by G. H. PARKER). *Biological Labs., Harvard Univ., Cambridge, Mass.*

It is well known that adrenal cortical hormones are necessary for normal lactation in experimental animals, but their relation to the development of the mammary gland has been equivocal. In the experiments reported here, immature rats were ovariectomized, pretreated with 1 μg. estradiol daily for 12 days, and adrenalectomized on the thirteenth day. Daily injections of 1 μg. estradiol, 1 mg. progesterone, and 90 G.P.U. relaxin, or of 1 μg. estradiol and 5 mg. progesterone, were begun on the day of adrenalectomy. Animals were autopsied after 7 or 12 days treatment, mammary glands were removed, fixed, measured, and stained as whole mounts. This treatment produces well-developed glands with alveoli in non-adrenalectomized controls. However, duct growth was impaired in the adrenalectomized rats, while alveolar growth was normal, compared to the controls. Maintenance with either 1% NaCl, 1% glucose in their drinking water, or with eschatin beginning on the day of adrenalectomy failed to restore the response of the gland to injected steroids and relaxin. Maintenance with daily doses of 0.5 mg. DCA, dehydrocorticosterone acetate, or cortisone acetate, resulted in normal duct growth. It is concluded that the adrenal cortex exerts a differential effect on mammary growth, that duct growth is more sensitive to a lack of adrenal hormones than is lobular-alveolar growth.

Potassium tolerance following irradiation. WILLIE W. SMITH. *Natl. Insts. of Health, Bethesda, Md.*

Functional activity of the adrenal cortex after irradiation has not been adequately studied, nor is its role in the development of radiation sequelae well understood. Since biochemical changes are not always subject to exact physiological interpretation, we have attempted to apply functional tests of the hypothesis that the adrenal response after irradiation is a non-specific stress reaction. Cortical extracts increase and adrenalectomy decreases tolerance to intraperitoneally injected KCl. With the assumption that KCl tolerance reflects some aspect of adrenal cortical function, this tolerance was determined on *days 1* through *5* following an amount of radiation (700 or 800 r) that kills essentially 100% of our mice beginning about 4 days after irradiation. The lethal effect of the KCl was slightly diminished on the first two days and thereafter was increased as the mice became obviously sick from radiation. Starvation alone resulted in changes in KCl tolerance of the same magnitude. Since starvation is one component of the sequelae of lethal radiation, this alone may account for the result in KCl tolerance following irradiation. Tolerance of irradiated mice to injected histamine seems to follow the same pattern, but histamine kills less rapidly than KCl and the late end-point is not altogether reliable. The late decrease in tolerance may be due to an accumulation of KCl and histamine in the body fluids which is unrelated to cortical function. The opposite shift on the first two days suggests a hyperactive phase of the adrenal cortex, as has been repeatedly shown for non-specific stresses.

Action of levo-isomethadone during labor. FRANKLIN F. SNYDER. *Dept. of Obstetrics and Anatomy, Harvard Univ., Boston, Mass.*

Levo-isomethadone exerts a powerful influence upon the outcome of labor in rabbits following administration at the time of parturition. The effect upon the uterine expulsive mechanism is shown by the increase in incidence of stillbirths and by increase in the duration of labor when the fetuses are expelled through the birth canal instead of delivery by hysterotomy. Observations on the spontaneous birth of 550 fetuses following levo-isomethadone show clearly that increase in the dosage increases the risk of death for the fetus

during expulsion through the birth canal. Thus, following a single injection of 6 mg/kg. of levo-isomethadone intramuscularly at 31 days, fetal mortality increased to 18%, or almost twice that noted in uninjected controls, and labor was prolonged for a period of hours or days in 14% of the animals. At a total dosage level three times as great, i.e., 3 injections of 6 mg/kg. in the course of 24 hours, further increase in stillbirths was noted, amounting to 40%, and in 37% of the animals labor was prolonged. The rapid escape of the drug from the maternal circulation into the fetus is demonstrable by direct observation of breathing fetuses within the unopened uterus following laparotomy in a saline bath. Fetal respiratory movements were depressed or temporarily abolished immediately after intravenous injection of 1 mg/kg. of levo-isomethadone into the mother. (Aided by Research Grant, U. S. Public Health Service.)

Permeability of the human erythrocyte to sodium and potassium in vitro. A. K. Solomon (introduced by J. R. Pappenheimer). *Biophysical Lab., Harvard Med. School, Boston, Mass.*

Measurements of the permeability of the human erythrocyte to potassium *in vitro* have indicated that 1.87% of the cellular potassium is exchanged per hour, and that the activation energy of the process is about 11,400 cals/mol, in agreement with previous results of Raker *et al.* and Sheppard and Martin. The total amount of potassium crossing the cell membrane per hour per liter of plasma (2.0 mEq.) is independent of plasma potassium concentration up to 14 mEq/liter plasma. Lithium does not affect the rate of potassium exchange, but rubidium does. Preliminary measurements of the permeability of the human erythrocyte to sodium indicate that only about half of the cell sodium is freely exchangeable. 1.89% of the plasma sodium enters the cell per hour, and 66.9% (2.6 mEq.) of the freely exchangeable cell sodium leaves the cell per hour. The activation energy for the entrance of sodium is about 18,200 cals/mol, and the process is essentially independent of external concentration. When the glucose has been exhausted, the amount of exchangeable cell sodium increases appreciably.

Various forms of actomyosin superprecipitation. Samuel S. Spicer. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

Szent-Györgyi showed that adenosinetriphosphate (ATP) induces contractile precipitation (superprecipitation) of a solution of the muscle proteins, myosin and polymerized actin (F-actin), at a particular KCl concentration. This reaction, because of its probable importance in muscle contraction, has been further investigated. F-actin and myosin in the presence of ATP and KCl form a precipitate which contracts to a small plug, but the degree of contraction varies with the order of mixing components and the actin-to-myosin ratio. With a large excess of ATP, F-actin and myosin form a flocculate or fine granular precipitate instead of a contracted plug. Added Mg at 10^{-5} to 10^{-6} M causes formation of a non-contractile flocculate. At 10^{-1} to 10^{-4} M, Mg delays but does not prevent appearance of a contracted plug. Mg decreases and Ca increases by severalfold the amount of ATP required to induce superprecipitation. In the presence of Mg over the range of concentration in which the Mg effect was studied, added Ca had no effect, even up to a concentration 10 times that of the Mg. Adding non-polymerized actin (G-actin) to a myosin-ATP solution or myosin to a G-actin-ATP solution gives a fine, granular, nonsettling precipitate if a recently-prepared myosin is used. An aged myosin preparation, or one kept a few hours at 26°C., forms a slowly contracting, reticular plug under such conditions. G-actin added to a myosin solution a moment before introducing ATP yields a loose plug or a non-contractile flocculate.

Enzymatic adaptation in non-viable cells. S. Spiegelman, L. S. Baron* and H. Quastler.* *Depts. of Bacteriology and Physiology, Univ. of Illinois, Urbana.*

Investigations in recent years on enzymatic adaptation have established that this phenomenon can be observed with non-dividing suspensions of viable cells. Sporadic attempts early in the history of the problem to simplify the experimental system further by employing dead cells were not successful. A systematic reinvestigation of this problem was undertaken. The adaptive enzyme systems formed in response to maltose and galactose in yeast (Strain K, a representative of *S. cerevisiae*) were employed as test systems. Capacity to adapt was followed manometrically by the two-cup method. The onset and further increase of aerobic fermentation of the adaptive substrate by preparations suspended in M/15 phosphate buffer at pH 4.5 could thus be followed continuously. Viable counts were made by the usual spread-plate methods supplemented by direct microscopic examination of clone size. None of the usual methods of cell destruction (freezing and thawing, grinding with abrasives, lysis with toluol, ethyl acetate, etc.) led to a separation of viability from adaptability. Ultra-violet and nitrogen mustard treatment gave some suggestion that non-viable cells could adapt. With both agents, maximal dissociation occurred at dosages yielding kills of 88%, at which capacity to adapt was decreased by 35%. Higher dosages of either agent resulted in com-

plete loss of adaptability. A clear cut separation of adaptability from viability was however achieved with x-rays. Dosages resulting in a 99.9% loss in viable cells did not decrease the capacity to adapt to either galactose or maltose. The lag, preceding the period of rapid enzyme appearance was somewhat longer (20–40 minutes) in x-ray killed cells. However, the rates of enzyme formation were about the same as normal and the final levels of enzyme activity achieved were invariably higher. The kinetics of adaptation were not modified by lethal dosages of x-rays, being of the usual autocatalytic type. (Aided by a grant from the National Cancer Institute of the U. S. Public Health Service.)

Temperature and the erythrocyte count of the goldfish. W. A. SPOOR (introduced by C. K. WEICHERT). *Dept. of Zoology, Univ. of Cincinnati, Cincinnati, Ohio.*

The fact that fish undergo temperature acclimatization is well established, but the mechanism by which this is accomplished is unknown. In view of the effect of temperature upon the metabolic rate of a fish, as well as upon the solubility of oxygen, there is reason to suspect that the erythrocyte count may increase as the temperature is raised. In order to test this possibility, erythrocyte counts were made on sibling goldfish subjected to different temperature conditions for a period of about 2 weeks. Fish which had been maintained at 12° to 14°C. for several months were divided into 3 groups, one of which was kept at temperatures between 13° and 14.8°C., another cooled and maintained between 4.8° and 7°C., and a third warmed gradually over a period of 10 days to 30°C. and held at this temperature for 4 days. All 3 tanks were aerated vigorously to maintain a high oxygen tension. The erythrocyte counts for each group varied considerably from one individual to the next, to the extent that the results must be interpreted with caution; nevertheless they indicate that the erythrocyte count is higher at high temperature than at low. The erythrocyte counts, in millions/mm.^{3}, ranged as follows: for fish brought to 30°C., 1.72–2.62, with the majority in excess of 2 millions; for fish held at 13° to 14.8°C., 1.26–1.91, with 1 dubious count of 2.02 millions. The erythrocytes of about half the fish in the 4.8° to 7°C. group were so nearly colorless that but few satisfactory counts could be obtained. These counts, however, were similar to those of fish held at 13° to 14.8°C.

Single unit activity in spinocerebellar tract in cats. JAMES M. SPRAGUE (introduced by WILLIAM W. CHAMBERS). *Dept. of Anatomy, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Activity of single fibers in the lateral funiculus was studied, using steel microelectrodes, electrolytically sharpened. Following movement of hairs, pressure on and stretching of muscles and tendons in hind leg and tail, and electrical stimulation of minute cutaneous and muscle nerve twigs in the hind foot, abundant activity was recorded from various electrode placements in the ipsilateral, lateral funiculus of segments T-8, 9 and 10. Responses to tactile and proprioceptive stimulation were present in both the deep and superficial parts of the lateral funiculus. Effective electrical stimulation was given by single, square-wave pulses. The threshold voltage of 0.1–0.15 was reached at a pulse duration of 0.5 msec. The activity of a single, central unit was frequently recorded from one electrode placement following both tactile and muscle-proprioceptive stimulation in the hind foot. It is not known whether this activity was from the same fiber in both types of stimulation. However, this type of activity in neighboring fibers was assumed to be relayed through a single nucleus, Clarke's nucleus, which projects ipsilaterally to the cerebellum. No contralateral activity was found at any depth nor in any part of the lateral funiculus following stimulation of nerve terminals, twigs, large nerves or dorsal roots. It is therefore concluded that the border cells of Cooper and Sherrington which have a contralateral projection to the brain stem and cerebellum via the lateral funiculus are not under the influence of activity coming from the periphery.

Effects of corticoids and ACTH on fluid exchange, blood pressure, glycemia; cholesterolemia and atherogenesis in chicks. J. STAMLER,* R. PICK* and L. N. KATZ. *Cardiovascular Dept., Med. Research Inst., Michael Reese Hospital, Chicago, Ill.*

Recent widespread therapeutic utilization of ACTH and adrenal corticoids compels careful study of their chronic effects on cardiovascular structure and function, particularly since they have been previously implicated in the pathogenesis of hypertension and vascular scleroses. Chronic exhibition of DCA to normal cockerels (1–4 mg. daily) induces cardiac hypertrophy, polydypsia, hyperpiesis, adrenal hypoplasia, decreased androgenic activity, and moderate nephrosclerosis. DCA-induced polydypsia and blood pressure rise are intensified by addition of 1% NaCl to the mash; neither supervene in DCA-treated birds on a low Na diet. DCA exhibition fails grossly to influence glycemia or cholesterolemia in chicks on mashes with or without a cholesterol supplement. Correspondingly gross atherogenesis is not significantly influenced by DCA in birds fed only plain mash. However, in cockerels given

a mildly atherogenic supplement of cholesterol (0.25%), which induces minimal hypercholesterolemia and organ lipidosis, concomitant DCA exhibition increases the incidence and severity of atherosclerosis. Possible mechanisms of these DCA effects will be discussed and compared with the somewhat different findings in cockerels treated with ACTH, ACE or cortisone.

Modification of cerebellar activity by various drugs. JACK STEELE* AND RAY S. SNIDER. *Dept. of Anatomy, Northwestern Univ. Med. School, Chicago, Ill.*

Electrical activity of the cerebellum of the rabbit was recorded over a period of several hours and the action of the following drugs observed upon it; apomorphine, Benadryl, alcohol, aspirin, Artane, atropine, bulbocapnine, Benzedrine, benzimidazole, cocaine, Coramine, Dilantin, Dramamine, D.D.T., ephedrine, triple bromide, mesantoin, panparnit, quinine, Tridione, and scopolamine. The following drugs *decreased* the amplitude of cerebellar frequencies below 100/second; atropine, bulbocapnine, Dramamine, D.D.T., mesantoin, and scopolamine. The following drugs decreased the amplitude of cerebellar waves faster than 100/second; bulbocapnine, Dilantin, and scopolamine. Drugs which *increased* the amplitude of cerebellar waves were; (above 100/second) Benadryl, alcohol, cocaine, Tridione; (below 100/second) alcohol, ephedrine, bromide, panparnit, and Tridione. The following drugs either produced no effect or an equivocal one; apomorphine, aspirin, Artane, Benzedrine, benzimidazole, Coramine, and quinine. In each case, an adult human dose was administered to a 2 kg. rabbit. Bipolar pickup electrodes were placed upon various cerebellar and cerebral areas. The electrical activity was amplified by means of a Grass Model III resistance-capacity coupled amplifier and recorded photographically by means of sensitive loop galvanometers.

Effect of blood and plasma transfusion on the bleeding time in psuedohemophilia. MARIO STEFANINI AND LEDA ZANNOS (introduced by JOSEPH H. PRATT). *Ziskind Lab., J. H. Pratt and New England Center Hosps., and the Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Inability to contract after injury of abnormal capillary structures is considered responsible for the hemorrhagic tendency of psuedohemophilia (von Willebrand's disease), a familiar hemorrhagic diathesis characterized by prolonged bleeding time, all other tests for the investigation of known factors of hemostasis giving normal results. We have studied 8 patients of this disease. During observations on the effect of several substances on the hemorrhagic tendency, a reduction, although not to completely normal values of the bleeding time (Duke and Ivy's methods) followed the transfusion of whole blood or plasma, in direct proportion to the amount administered. Fresh platelet-rich blood and plasma were highly effective, reducing bleeding time to almost normal levels. Storage of blood and plasma under ordinary refrigeration was accompanied by progressive loss, until complete disappearance in 5–10 days, of their active fraction. The effect of blood and plasma on bleeding time was temporary, original values being restored in 4–12 hours. Two patients were subjected to minor operative procedures without excessive bleeding after transfusion of fresh plasma. Observations are in progress for the identification of the plasma fraction responsible for the effect observed and the establishment of its relationship with the active platelet-born vasoconstrictor substances found in shed blood by O'Connor and others. Fresh blood and plasma possess a similar temporary beneficial effect on the bleeding time in thrombocytopenic purpura, even when failing to raise the platelet count.

Effects of water or milk on colon activity and flatus composition in man at altitudes. F. R. STEGGERDA, W. C. CLARK* AND I. E. DANHOF.* *Dept. of Physiology, Univ. of Illinois, Urbana.*

One can record the effects of drinking water or milk on colon activity at various altitudes by inserting one end of an open tipped catheter into the empty lower colon about 6–8 inches beyond the anal sphincter, and attaching the other end to an electronic air recording pressure pick-up unit placed outside the decompression chamber. By means of a T-tube attachment in the catheter one can also either inject room air or collect flatus samples for analysis whenever desired. The results indicate that, for at least 45 minutes after 300 cc. of room air are injected into the lower colon, there is a progressive increase in colonic activity with increased altitude within the range studied (ground to 25,000 ft.). The activity following the drinking of 450 cc. milk is more than that with a similar amount of water. Similarly, progressive changes were recorded in the CO_2 and O_2 composition of the collected flatus samples, the average CO_2 concentrations reading 30.8% and O_2 decreasing to 6.8%. In some cases the CO_2 was found as high as 40% and O_2 as low as 2%. It is suggested that these changes in activity and chemical composition of the collected flatus can be accounted for on the basis of an increase in the active transfer of gas from the small intestine into the colon with altitude, rather than entirely on diffusion equilibrium between body cells and the colon.

Measurement of radiant heat load on man in outdoor environment. ALICE M. STOLL (introduced by E. F. DUBOIS). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

Measurement of radiation and convection variables in the outdoor environment were made with the pan-radiometer. Radiation measurements included the solar and infrared components, determined separately. Convection was determined from measurements of air temperature and velocity. Determinations of direct solar radiation and radiant temperature of the surroundings were made under various environmental conditions and found to be in agreement with those obtained by direct measurement. Measurements of the radiant temperatures of the surroundings outdoors and indoors were also found to be in agreement. Daily variations in direct solar radiation, the radiant temperature of the surroundings excluding the sun, and the radiant temperature of the surroundings including the sun were measured. From these data the operative temperatures in the sun and shade were computed and compared with the air temperature. It was found that the operative temperature differed from the air temperature by 2-3°C. in the direction of the radiant temperature. The radiant heat load on man and the operative temperature in Death Valley were determined from measurements made with the pan-radiometer by the Environmental Physics Unit of the Office of the Quartermaster General, U. S. Army. Comparison of these data with the observations made in New York City showed that with approximately the same intensity of solar radiation in both environments the operative temperature in the desert was about 30°C. higher, and radiant heat load as much as 25 times that experienced in this city.

Accelerating effect of Pyromen in contrast to inhibitory effect of cortisone on the Arthus phenomenon in rabbits. EDWARD G. STUART (introduced by WILLIAM F. WINDEL). *Dept. of Anatomy, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Twenty-four male albino rabbits of approximately 1.5 kg. weight were sensitized with crystalline hen's egg albumin in daily intracutaneous injections containing 0.5 mg/kg. of egg albumin nitrogen. Eight served as controls, 8 received 10 mg. cortisone daily intramuscularly, and 8 received 10 gamma Pyromen (Baxter) daily intravenously. Treatment continued for 35 days during which time precipitin tests for serum antibody were made on appropriate days. Seven cortisone-treated animals showed no Arthus reactions, but one gave a positive reaction on the 23rd and succeeding days of the experiment. The control animals showed their first Arthus reactions about the 7th day. The reactions became pronounced about the 10th day and continued to appear at intracutaneous injection sites throughout the experiment. The Pyromen treated rabbits showed their Arthus reactions initially on the 4th or 5th days. The lesions passed rapidly through the usual course, markedly exceeding those of the controls in respect to size, degree of inflammation, hemorrhage, suppuration and necrosis. Scab formation was rapid but in most instances scabs were resorbed rather than sloughed off as in the controls. Precipitin titers, absent in nonreacting cortisone animals, appeared earlier in the Pyromen animals and were at least 50% higher than in the controls. These effects of Pyromen may be correlated with its known property of stimulating the leucopoietic, phagocytic and reticular elements of the body. (Aided by a grant from Baxter Laboratories, Inc.).

Effect of certain anticonvulsants on QO_2 of dog cerebral cortex. D. L. STUMPFF AND R. W. BELKNAP (introduced by H. C. STRUCK). *Dept. of Physiology and Pharmacology, Creighton Med. School, Omaha, Nebr.*

Previous studies on Tridione have been extended to include Dilantin and Mesantoin, and results with Tridione have been repeated and confirmed (Federation Proc. 9: 123, 1950). It was again found that Tridione inhibited the motor cortex significantly, but not the sensory area where the Tridione concentration was 0.035 M. In addition, 346 slices were obtained from the sensory cortices and 243 from the motor cortices of 29 dogs, approximately equal numbers of slices being obtained from each hemisphere of each dog. Half of the slices from each dog were placed in Krebs-Ringer-Phosphate-glucose solution, and half in the same solution, fully, half, or quarter saturated with Dilantin (free compound) or Mesantoin, respectively. The QO_2 of the slices was followed for three hours at 38°C. using oxygen as the gas phase. The mean inhibition of QO_2 of motor and sensory cortices by Dilantin in all concentrations used was the same within experimental error: saturated: motor, 20%; sensory, 24%; half-saturated: motor, 10.0%; sensory, 12%; quarter-saturated: motor, 5.9%; sensory, 3.9%. The inhibitions in saturated and half-saturated Dilantin were statistically significant. The mean inhibition of QO_2 of motor and sensory cortices by Mesantoin in all concentrations used was likewise identical within experimental error: saturated: motor, 26%; sensory, 28%; half-saturated: motor, 12%; sensory, 12%; quarter-saturated: motor, 8.4%; sensory, 8.6%. Again only inhibitions at the two higher concentrations are significant. These results indicate that Tridione probably exercises

its inhibitory effect in a manner different from Dilantin and Mesantoin.

Effects of estrogens and thyroxine upon plasma proteins, blood volume and hematocrit of the fowl. PAUL D. STURKIE. *Lab. of Avian Physiology, Rutgers Univ., New Brunswick, N. J.*

Plasma albumin and globulin were determined in hens by the biuret method of Gornall *et al.* (1949); 2.5 mg. of T-1824 was injected and after a mixing time of 3 minutes (PINO AND WEISS, 1950) blood samples were taken and plasma volumes determined. Dienestrol diacetate (DD) was fed at the level of 0.02% of the feed (av. of 20 mg. of DD/bird/day) for 4 weeks (*A*) and then at 0.04% to the same birds for 2 weeks (*B*). *Treatment B* increased the plasma albumin (2.30 gm. %) and globulin (5.20) significantly over the control values (1.94 and 3.20 gm., respectively) but did not appreciably change the A/G ratio (0.61) from that of the controls (0.60). *Treatment A* did not increase plasma proteins significantly. In another group of hens receiving 0.04% DD, total protein was increased to 8.12 gm. % with no change in A/G. When these birds continued to receive DD plus 1 mg. of D,L-thyroxine daily, per bird, intramuscularly, for 7–10 days, plasma albumin (1.97) and globulin (2.67) dropped below control levels (2.50 and 2.97, respectively). The decrease in albumin, but not globulin, was significantly below the control values. The A/G ratios for the control and thyroxine-treated birds were 0.85 and 0.74, respectively. Plasma volume and cell volume were not affected appreciably by the estrogen or thyroxine.

Plasma free and total urinary amino acids of hepatectomized dogs. MURIEL SVEC* AND SMITH FREEMAN. *Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

Microbiological estimations of 16 individual amino acids and the chemical estimation of glycine and alanine were carried out on the plasma and urine of dogs before and after hepatectomy. The plasma free amino acids were estimated at 4-hour intervals for the first 12 hours after hepatectomy. Each plasma amino acid was studied in from 4–11 dogs. The plasma concentration of glycine, histidine, phenylalanine, glutamic acid and lysine increased after hepatectomy. while that of methionine, tryptophane, leucine, isoleucine, arginine and valine decreased after hepatectomy. Cystine, alanine, tyrosine, serine, threonine and proline either did not change significantly or gave inconclusive results. The intravenous administration of 0.3 gm/kg/hour of glucose for a 12-hour period to fasting animals prior to hepatectomy caused an increase in the urinary excretion of all of the amino acids studied. A further rise in excretion to levels two to four times the glucose control values occurred for most amino acids following hepatectomy. Glycine excretion was slightly less after hepatectomy as compared to the glucose control period. There was only a slight increase in histidine excretion. The other amino acids arranged in relation to their relative increased urinary excretion following hepatectomy are: Glutamic acid, asparticacid, serine, lysine, alanine, leucine, valine, threonine, arginine, proline, phenylalanine, tyrosine, isoleucine, tryptophane, cystine, methionine, histidine.

Relation of intrarenal pressure to blood pressure and to perinephritic hypertension. H. G. SWANN AND J. M. PRINE.* *Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.*

In dogs, the intrarenal pressure (IRP) was found to be related to the blood pressure (BP) thus: IRP = 9.4 + .22 BP. When this statement was applied to Selkurt's data (*Am. J. Physiol.* 147: 537, 1946) on renal blood flows (RBF), the empirical formula $RBF = 2.4\left(\frac{BP}{IRP} - 1\right)$ was found to give an exact description of the relationship of the three variables. The anomalous blood pressure–blood flow relation in the kidney may therefore, it is probable, be ascribed primarily to the effect of blood pressure on intrarenal pressure. These formulations also quantitate the hypothesis that the hypertension of perinephritis is due to a decreased renal blood flow. In the disease, experimentally produced in dogs, the two pressures were measured. The intrarenal pressure increased from the normal of 25 up to 50–60 mm. Hg. Since for a normal flow of 5 cc/min/gm. the blood pressure must rise approximately three times faster than the intrarenal pressure, the blood pressure should rise in the disease to about 150 mm. Hg. This in fact takes place, for a rapid elevation in diastolic pressure to 130–160 mm. Hg was observed. The course of the disease can only end, if the intrarenal pressure continues to rise, in irreversible damage to the circulatory or renal system.

Histochemical alterations in denervated skeletal muscle of mice. RICHARD H. SWIGART* AND W. LANE WILLIAMS. *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Denervation was accomplished by gluteal section of sciatic nerves, abdominal section of femoral nerves, or both. Four to 6 months later denervated muscles showed *1*) reduction in trypsin-digestible basophilia (no ribonucleic acid was demonstrable in normal or denervated muscle) and in acidophilia (Hayden acid dye method at pH 1–2 for certain proteins); and *2*) a very great increase in substances reacting positively to periodic acid-Schiff's

reagent (PA-S). The latter reaction was not decreased by prior removal of glycogen but was reduced by trypsin-hydrolysis indicating that the PA-S-positive material was a protein-polysaccharide. The PA-S reaction of normal muscle (glycogen excluded) is very slight while that of dense fibrous (collagenous) tissue is quite strong. In denervated muscle this reaction was greater, as to distribution and intensity, than that of tendon, ligament or muscle fascia. Within denervated muscle the occurrence and location of PA-S-reacting substance was closely correlated with positive staining reactions (staining by light green) for connective tissue following use of the Masson stain for connective tissue and muscle.

Pitressin and Mercuhydrin in relation to electrolyte excretion of patients with diabetes insipidus. ROBERT TARAIL AND FRANK M. MATEER (introduced by PAUL L. MCLAIN). *Dept. of Research Medicine, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Three adults and one child with authentic, untreated diabetes insipidus received 0.5–1.0 cc. of Pitressin (20 U.S.P. pressor units per cc.) intramuscularly. Their intakes of sodium chloride had not been restricted. Control and post-injection urines were collected for 4–5 hours at ½- to 1-hour intervals. Pitressin caused a sharp but ephemeral increase in specific gravity and diminution of urine flow within 1 hour while 240–300 cc. of water per hour were ingested. Excretion rates of sodium, chloride, and potassium were not significantly altered, aside from occasional transient fluctuations, despite the fact that Pitressin is said to be both natriuretic and chloruretic. Accelerated sodium and chloride excretion, however, followed intravenous Mercuhydrin in two of the patients under similar circumstances thereby indicating potential renal response. The mercurial also produced augmentation of urine flow and perhaps of potassium excretion, but little alteration of specific gravity. In another comparable experiment, Pitressin injected while the action of Mercuhydrin was increasing only partially prevented the water diuresis. Specific gravity and excretion of sodium, chloride, and potassium rose during combined Pitressin-Mercuhydrin action. Pitressin alone did not change the tendency of sodium and chloride excretion to fall, nor affect potassium excretion during water deprivation in one subject. Pitressin and water-loading during intensive cortisone therapy of one patient produced effects indistinguishable from those before cortisone.

Relationship between basal cardiac output and body composition with special reference to obesity. HENRY LONGSTREET TAYLOR, JOSEF BROZEK AND ANCEL KEYS. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

Total body fat was estimated from the specific gravity of the body on 34 young men between the ages of 18 and 30. The correction of the submerged weight for the residual volume of the lungs was made by direct determination of the residual volume in 21 of the men who varied most in fatness. Body weights varied from 51.8–132.5 kg. and body fat from 0.4–33.7%. Four cardiac output measurements were made on each individual by the acetylene method and the values were corrected to direct Fick levels by the regression equation given by Chapman *et. al.* (*J. Clin. Investigation*, Vol. 29). Regression equations obtained from the above data showed that basal cardiac output can be predicted from body weight with a standard error of ±0.75 liters, from surface area with a standard error of ±0.70 liters, from fat weight with a standard error of ±0.86 liters and from fat-free weight with a standard error of ±0.58 liters. The weight of fat and fat-free body weight are related by a correlation coefficient of 0.46 ($r = 0.44$ at $P_{0.01}$). The correlation coefficient between cardiac output and gross weight was 0.52, between cardiac output and surface area 0.60, between cardiac output and weight of fat 0.16 and between cardiac output and fat-free body weight 0.74. It is concluded that the blood flow through fat is not an important factor in determining the resting cardiac output and that the increased cardiac output observed in obesity is primarily related to increased fat-free tissue.

Changes in peripheral circulation induced by vasoactive drugs acting upon cerebral chemoreceptors. ROBERT D. TAYLOR AND IRVINE H. PAGE. *Cleveland Clinic Fndn., Cleveland, Ohio.*

We have tested the effect on the peripheral circulation of circulating adrenaline, noradrenaline, barium chloride, nicotine, prostigmine, histamine, and acetyl-B-methyl choline through the brains of dogs connected to the body only by the spinal cord. The blood supply was furnished by a donor or by perfusion pump. If the drug was vasopressor in intact animals, it caused sharp and often prolonged vasodilatation and reduction in arterial pressure in the body of the test animal when injected into the isolated cerebral circulation. Vasodepressor drugs caused the opposite. It was shown that both the vasoconstrictor and dilator impulses pass over the sympathetic system by performing surgical sympathectomy and ganglionectomy, section of the antero-lateral columns, and ganglionic blockade with tetraethylammonium chloride. Resection of the carotid sinuses and vagus nerves failed to block the responses initiated in the brain. The potent cerebrovascular dilator, CO_2, had no appreciable effect upon the arterial pressure when given in concentrations as great as 70% of the

respired air of the donor dog. Since the vascular responses occur in absence of known baroreceptors and cannot be elicited by the vasodilator CO_2, we believe the drugs act upon previously unrecognized chemoreceptors within the brain.

Effects of pituitary growth hormone and cortisone administration in vivo on ketogenesis by surviving liver slices obtained from hypophysectomized rats. JAY TEPPERMAN AND JANET M. DEWITT.* *Dept. of Pharmacology, State Univ. of New York Med. Center at Syracuse.*

Bondy and Wilhelmi (*Federation Proc.* 8: 185, 1949) discovered that liver slices obtained from hypophysectomized rats produced significantly fewer ketone bodies than did slices from intact rats. The present study was undertaken to determine whether or not purified pituitary growth hormone would correct this metabolic defect. After Bondy's and Wilhelmi's finding had been satisfactorily confirmed hypophysectomized rats were injected with 3 mg. of growth hormone (Armour), sacrificed 3 hours after injection, and ketogenesis by their liver slices was measured over a period of 2 hours by the method of Greenberg and Lester. A Krebs Ringer phosphate buffer of pH 7.4 was used and oxygen was the gas phase. During the 2 weeks following operation liver slices from growth hormone-treated hypox. rats made more ketone bodies than did those from untreated, operated rats (100 ± S.E. 1.9 μg/100 mg. wet tissue/2 hours versus 131 ± S.E. 5.3 μg/100 mg. wet tissue/2 hours, as acetone). Experiments done on the 31st, 37th, and 40th days post-operatively revealed no significant difference between the performance of slices obtained from the treated and untreated groups. In other experiments done after the 50th post-operative day pre-treatment with cortisone and treatment with growth hormone resulted in a highly significant difference between the treated and control groups (121 ± S.E. 3.6 and 87 ± S.E. 2.4, respectively). The results of concurrent measurements of the oxygen consumption of the liver slices, liver fat content and blood ketone concentration will be presented.

Temperature sensation in ventilated suits. R. THAUER, H. R. GREIDER* AND J. V. CORREALE.* *Aero. Med. Equipment Lab., Philadelphia, Pa.*

In forty-two 3-hour experiments on the same subject the effect of ventilation of an airtight suit on temperature sensation and temperature regulation was studied. The variables were: room temperature, flow rate and temperature of ventilating air, and insulation of the suit. The measurements included: rectal temperature, skin temperature, suit-surface temperature, total weight loss, water loss from skin and respiratory tract, temperature of expired and inspired air, metabolism, heat loss or gain of ventilating air, and environmental conditions. In addition, the comfort votes and temperature sensations of the subject were recorded. Plotting average skin temperatures against environmental temperature in such experiments in which the subject was kept comfortable by ventilation showed that the sensation of comfort is not related to a specific average skin temperature, as Yaglou found in experiments with varying insulation. With rising environmental temperature (0–35°C.), the average skin temperature correlated with comfortable sensations increased from 32.5 to 35.5°C., and the water loss from the skin more than doubled (from 20–50 gm/hour and more). The subject, in other words, felt cooler than his skin temperature and water loss indicated, when the environmental temperature was high, while he felt warmer than he actually was in a cold environment. The relationship of skin temperature and water loss to environmental temperatures and subjective comfort changed with changing insulation of the suit. The explanation for these observations is the paradoxically increased or decreased temperature at the inlets of the ventilated suit (feet, hands and face) in cold or warm environments respectively.

Myenteric reflex in dog's duodenum. J. E. THOMAS. *Jefferson Med. College, Philadelphia, Pa.*

Two balloons 5 cm. apart are inserted through a chronic fistula in a dog's duodenum so as to lie about 20 cm. below the pylorus. The balloons are connected to water manometers. Injection tubes are provided one to end in the area between the balloons and one above the upper balloon. If 2 cc. of n/10 HCl, 2% soap solution or bile are injected between the balloons there follows an increase in tone and in amplitude of rhythmic contractions over the upper balloon and the opposite effect over the lower balloon. If the injection is made above the upper balloon there is inhibition over both balloons. The experiment succeeds only with moderate stimuli and if activity of moderate grade is present; presumably these are 'normal' or usual conditions. The response is presumed to be a myenteric reflex because of the nature of the stimulus and the demonstration by previous investigators of similar responses in the absence of the extrinsic innervation.

Effect of alterations of renal arterial pressure on sodium and water excretion. DAVID D. THOMPSON* AND ROBERT F. PITTS. *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

It has been shown by a number of investigators that lowering the mean renal arterial pressure results in a reduction in the excretion of sodium and water. Our experiments confirm and extend these findings. In female dogs, anesthetized with

chloralose, a balloon catheter was introduced through one femoral or common carotid artery and positioned above the renal arteries. Physiological saline was infused for 2–3 hours until the urine flow was greater than 5 cc. per minute. Thereafter collections of blood and urine were made at varying renal arterial pressures. Creatinine, PAH, sodium, and potassium clearances were measured. Eight experiments performed in this manner yielded results in agreement with the findings of others. At mean pressures of 90 mm. Hg or below glomerular filtration rate and renal blood flow were reduced. The excretion of sodium and water was reduced to a proportionately greater extent; hence the percentage reabsorption of sodium increased. Experiments are now under way to determine whether these phenomena result from intrinsic responses by the kidney or are the result of hormonal or neural mechanisms acting upon the kidney in response to altered hemodynamics. Results in one experiment on an adrenalectomized animal reveal the responses to be qualitatively similar to those found in normals. (Supported by a research grant from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service).

Ultrastructure and function in nerve. JULIAN M. TOBIAS AND SIDNEY SOLOMON.* *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Continuing studies on the effects of electrical currents on axone structure will be described, and certain non-electrical, physical events which, primarily from polarization experiments, seem likely as accompaniments of excitation will be called to attention.

Clot accelerating activity of euglobulin fractions from normal and hemophilic plasma. L. M. TOCANTINS, R. R. HOLBURN* AND R. T. CARROLL.* *Div. of Hematology, Dept. of Medicine, Jefferson Med. College and Hosp., Philadelphia, Pa.*

Citrated plasma collected from normal and hemophilic subjects was diluted with cold distilled water and the euglobulins precipitated at pH 6.0 with 1% acetic acid. The sediment was dissolved in 0.85% NaCl and its pH adjusted to 7.4. The euglobulins separated from plasma diluted to a concentration of 10–20% have less clot accelerating effect on normal and hemophilic plasma than those made from concentrations of 2% or below. Hemophilic euglobulin prepared from 10% plasma has little activity, while euglobulin prepared from 10% normal plasma has a strong clot accelerating activity. As more diluted plasma is used for the preparation of the euglobulins, the activity of the hemophilic euglobulin gradually increases and eventually equals that of the normal euglobulin. When a highly active normal euglobulin solution is incubated with citrated normal plasma before recalcification, much of its activity is lost within 30 minutes; when incubated with hemophilic plasma, it loses all activity within 10 minutes. The difference between normal and hemophilic plasmas in their response to hemophilic euglobulin can be eliminated by appropriate dilution of the plasmas to about 5% concentration. Euglobulin solutions prepared from hemophilic plasma at 1% or less concentration accelerate the coagulation of hemophilic blood *in vitro* and *in vivo* to an essentially similar degree as when the euglobulin solution originates from normal plasma at 10% concentration.

Analysis of 'psychomotor' seizure discharges in rabbits. J. E. P. TOMAN. *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

Seizures consisting of about 20 seconds of automatism without reflex loss were induced by brief bursts of 6/second stimulation through bipolar epidural electrodes. Test stimuli at the same site were used to study threshold and wave-form changes in evoked cortical potentials, which were recorded from local and distant leads by ink-writing and cathode-ray oscillographs during control, shock, seizure and recovery periods. The normal local cortical response from various sites could be resolved into the following sequence of components: direct, monosynaptic, dispersed synaptic, slow wave and spindle. The development of a local seizure was characterized by the appearance of high-frequency (1000/second) low-voltage spikes and a large increase in synaptic response. Surface-negative seizure waves of 3–10/second frequency and 40 msec. duration appeared to develop from the dispersed synaptic response, and were recorded at a distance as longer positive waves. Following each attack, high frequency activity and synaptic facilitation declined together, leaving thresholds moderately elevated for several minutes. Of several drugs tested, Phenurone appeared to prevent seizures by a general threshold-raising action. These observations suggest that excessive low-frequency stimulation may initiate focal high frequency background activity which facilitates synaptic transmission locally and permits the development of a self-sustaining self-limiting relaxation-oscillator. The resultant synchronous slow spikes project to other cortical areas, causing behavioral automatisms of psychomotor type.

Effect of cortisone and ACTH on the threshold of convulsions induced by pentamethylene tetrazol. CLARA TORDA AND HAROLD G. WOLFF. *Depts. of Medicine (Neurology) and Psychiatry, New York Hosp.-Cornell Med. College, New York*

City and Kingsbridge Hosp. (V.A.), Bronx, New York.

Convulsive seizures can be precipitated in predisposed humans by repeated exposure to stressful situations. Also, lesser amounts of convulsant agents induce fits in such patients than in those not subject to stress. In experimental animals cortisone lowers the threshold of convulsions induced by electric shock. In the following the effects of cortisone and ACTH on the threshold of convulsions induced by pentamethylene tetrazol were investigated. Electroencephalographic studies were performed in curarized rats. Convulsions were induced by intravenous infusion of a solution containing 50 mg. of pentamethylene tetrazol in 100 cc. of mammalian Ringer's solution. Pentamethylene tetrazol was infused in an average speed of 1 cc/1 minute. Significant changes in the electroencephalogram occurred durinf the infusion of from 15–25 cc. Cortisone and ACTH were given either as single injection or were administered repeatedly in large amounts for 3 or 4 days. The amount of pentamethylene tetrazol necessary to induce convulsions evidenced by the changes in the electroencephalogram was less in animals injected with the hormones than in non-injected rats. The lowering of threshold was more marked after a single injection of ACTH than after the repeated large doses of ACTH.

Synaptic inhibitory action of adrenaline at parasympathetic synapses. CAROLINE TUM SUDEN AND AMEDEO S. MARRAZZI. *Toxicology Section, Med. Div., Army Chemical Center, Md.*

The synaptic inhibitory action of adrenaline described by Marrazzi (*Am. J. Physiol.* 127: 738, 1939; *J. Neurophysiol.* 10: 167, 1947) has been demonstrated by recording postsynaptic potentials of sympathetic ganglia. However, the phenomenon has been interpreted by him as evidence of a reciprocal relation between an adrenergic inhibitory mechanism and the cholinergic excitatory one present in sympathetic ganglia. A cholinergic excitatory mechanism is also thought to be present in parasympathetic ganglia and the possibility of an analogous reciprocal relation with an adrenergic mechanism has needed investigation. Since parasympathetic ganglia are in most cases imbedded in the effectors they innervate, this is ordinarily not feasible. Taking advantage of the fact that the ciliary ganglion is an exception in the parasympathetic system in that it has an accessible postganglionic nerve, it has been prepared for stimulation of the centrally sectioned preganglionic nerve and recording of action potentials from the peripherally sectioned postganglionic nerve when adrenaline reached it through its natural circulation. An inhibition by adrenaline similar to that observed in sympathetic ganglia has been found. Adrenergic inhibition has also been demonstrated at cerebral synapses (*Federation Proc.* 1: 57, 1942; *Am. J. Physiol.* 163: 1950) where acetylcholine excites. Thus adrenergic inhibition would appear to be a general phenomenon at cholinergic synapses.

Effect of omission of breakfast on amount of oxygen required by men past 60 years of age to perform work. W. W. TUTTLE AND KATE DAUM.* *Depts. of Physiology and Nutrition, State Univ. of Iowa, Iowa City.*

An experiment involving 8 men ranging in age from 62–83 years was performed to show the effects of the omission of breakfast on the amount of oxygen required to walk on a treadmill up a 2% grade at the rate of 2 mph. The procedure was to have all subjects eat a freely selected diet for a period of 3 weeks, so that we might learn something concerning their likes and dislikes, and to determine their approximate food requirements. After the period of free food selection, all subjects ate 3 meals per day, which consisted of prescribed diet, adequate in all respects, for a period of 4 weeks. During the 4-week period which followed, the procedure was the same, except breakfast was omitted. Twice each week, during both 4-week periods, the amount of oxygen required to perform the work was measured. A comparison of the amount of oxygen used to perform the work during the breakfast and no-breakfast periods showed that 7 of the 8 subjects required significantly more oxygen to perform the work during the no-breakfast period than during the time they were eating an adequate breakfast.

Effect of delayed additions of various substrates on respiration of excised brain tissue. DAVID B. TYLER AND MATILDA TYLER.* *Depts. of Physiology and Pharmacology, School of Medicine, Univ. of Puerto Rico, San Juan.*

It is well known that in the absence of a substrate the O_2 uptake of excised rat brain falls off rapidly. This study is concerned with the effect of delayed additions (intervals up to 5 hours after placing vessels in bath) of different substrates on the ensuing respiration. Glucose, lactate or pyruvate added to a minced brain in Ringer PO_4 stabilizes the O_2 uptake at the endogenous level found at about 30 minutes before the substrates are added, and it remains at that level for the duration of the experiment. Adding an excess of DPN or ATP along with the substrate raises the respiration to a level almost approaching the level of vessels containing the substrate from the start. In some instances with DPN the O_2 uptake falls off very rapidly again and is soon below the vessels where the substrate is added alone. Thus, in the

absence of any of the above substrates there occurs a loss in the capacity of the enzyme systems to oxidize the substrate. On the other hand, the delayed addition of succinate results in a higher O_2 uptake than that found for vessels containing it from the start and to which extra succinate is added to correct for concentration differences, indicating that the presence of this substrate inactivates some part of the enzyme system concerned with its oxidation.

Mechanism of action of the naturally-occurring lysins and inhibitors that are found in guinea pig liver extracts. DAVID B. TYLER AND MATILDA TYLER.* *Depts. of Physiology and Pharmacology, School of Medicine, Univ. of Puerto Rico, San Juan.*

Reference is made to a previous study showing that the difference in hemolytic activity between the fetal and adult liver preparations is principally due to differing inhibitory capacities rather than to differences in the concentration of the lysin (*Science* 112: 456, 1950). Saline extracts of fetal guinea pig liver allowed to stand alone or with added red blood cells for various intervals up to 3 hours, and then heated, lose their lytic activity. Such results do not permit the conclusion that the production of a heat stable lysin by a heat labile non-lytic agent is responsible for the hemolytic activity. Furthermore, the lysin molecule appears to enter into an inseparable complex with some component of the erythrocyte, or is otherwise 'used up' during the process of hemolysis. Recombining the lysin with various amounts of the naturally-occurring inhibitor from the adult liver cell (mitochondria fraction) results in almost the instantaneous establishment of equilibria, indicating that the lysin and its inhibitors behave in accordance with the mass action law to form an easily dissociable inhibitor-lysin complex.

Effect of thyroid hormone on respiration of cardiac tissue in albino rat. WILLIAM C. ULLRICK* AND WILLIAM V. WHITEHORN. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Effects of thyroid on cardiac activity have been widely noted, but details as to fundamental action are scarce. Investigation was therefore made into the respiratory metabolism of auricles and ventricles of hyperthyroid rats. Nine male albino rats were fed 100 mg. per day of USP desiccated thyroid for 16 days following which respiratory rates of cardiac tissues were determined by direct Warburg technique. Auricles were used whole; ventricles were sliced to 0.4 mm. thickness on a modified Stadie-Riggs microtome. Q_{O_2} determinations were made at 37.8 C. using Ringers-glucose-phosphate buffered solution at pH 7.4. Results were compared with rates obtained from 17 control animals. Hyperthyroid rats showed an average BMR 97% above the control average and suffered a 0.4% weight loss compared to 11% gain for controls. Hyperthyroid auricular respiration was increased 45% over normal while ventricular rate was increased 23%. No auricular-ventricular difference existed in controls, but hyperthyroid auricular respiration was 36% above that of hyperthyroid ventricles, the difference being highly significant. Results confirm the direct action of thyroid hormone on cardiac metabolism and indicate a greater susceptibility of auricular tissue to such action.

Effect of painful stimuli on intestinal motility of albino and hooded rats. EDWARD J. VAN LIERE, HELEN H. HESS* AND EDWARD J. FEDOR.* *Dept. of Physiology, West Virginia Univ. School of Medicine, Morgantown.*

A pair of albino rats (Wistar strain) or a pair of hooded rats (Long-Evans strain) which had been fasted for 24 hours and which were as nearly alike in weight and age as possible were given 2 cc. of a charcoal mixture by stomach tube. At the end of 10 minutes, one rat was placed in a wired compartment arranged so that painful electric stimuli could be given every 5 seconds; the control rat was put into an adjoining, but unwired compartment. Thirty minutes later they were taken out of the compartments and decapitated. The small intestine was removed, slit open and the distance the charcoal had traversed it measured. The painful stimuli, among other things, undoubtedly excited the sympathetic division of the autonomic nervous system, and it would be expected that the propulsive motility of the small intestine would be distinctly decreased. The results showed that the propulsive motility of the small intestine of the albino rat was significantly ($P - < .01$) decreased, whereas that of the hooded rats was not. The hooded rat is more closely related to the wild rat than is the albino. The wild rat is subjected to many environmental hardships and is more inured to painful stimuli than the domestic rat. In order to exist he must needs be alert and aggressive; these qualities probably have survival value. Because of the genetic background of the hooded rat, it may be less susceptible to external painful stimuli than the albino.

In vitro uptake of radiomethionine in normal tissue and carcinoma. LESTER VAN MIDDLESWORTH. *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

The 3-hour accumulation of S^{35} radioactive methionine has been measured *in vivo* and *in vitro* in various normal tissues and in fracture calluses in the rat. The uptake of radiomethionine is slightly greater *in vitro* than *in vivo*, possibly

because of minimal amount of carrier methionine *in vitro*. The *in vivo* results have been reported previously. Incubation (in Krebs-Henseleit solution containing 0.05 μc. radiomethionine) of 25–50 mg. of the following tissues of the rat resulted in the respective percentage ($\times 10^{-4}$) uptake of S^{35} μg. nitrogen: muscle 2, fibula 0.6, fibula fracture 2.2, duodenum 7.4, liver 10.5, pancreas 27.0. Further experiments were performed upon 50 mg. surviving biopsy samples of 4 cases of human bone tumors with appropriate controls and biopsy samples of 6 human cervix uteri. The control bones took up 1×10^{-4}% of the S^{35}/μg. N, the benign bone tumors accumulated 1.3×10^{-4}%. A malignant bone tumor took up 18×10^{-4}%. The control cervix tissue accumulated 2.7×10^{-4}% of the S^{35}/μg. N while 4 cases of cervical carcinoma took up 12×10^{-4}%. There is a possibility that this method might aid in determining protein synthetic activity of tissues and it might be a diagnostic aid in some types of carcinoma.

Thyroid depression by stress. LESTER VAN MIDDLESWORTH (with the technical assistance of MARY MARGARET BERRY*). *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

A study of I^{131} metabolism in anoxic rats has led to investigation of the function of the thyroid gland during different types of severe stress. Animals were raised on a low iodide diet and while undergoing various stresses 10 μc. of I^{131} were injected. After 24 hours radioiodine distribution between plasma protein bound, PBI^{131}/ml. plasma, and unbound, I^{131}/ml. plasma, was determined. I^{131} in thyroid and urine was also measured. Results are tabulated.

STRESS	NO. RATS	% OF INJECTED DOSE OF I^{131}			
		PBI^{131}/ml $\times 10^4$	I^{131}/ml $\times 10^4$	Thyroid	Urine
Control	10	0.15	0.018	37	45
Anoxic	7	0.015	0.42	6	31
Neph.	6	0.014	0.20	30	
Sham-oper.	4	0.07	0.015		27
Pinched-gut	5	0.04	0.10	23	43
Starvation	2	0.05	0.35	12	24
No Vitamin A	8	0.11	0.06	19	55
No Vitamin B_1	5	0.04	0.65	13	22
No Vitamin B_2	8	0.10	0.06	20	60
No Vitamin B_6	7	0.08	0.07	20	53

Disappearance rate of intravenous PBI^{131} was determined in controls, anoxic and nephrectomized rats; it appears to be similar in all groups. We believe these data indicate thyroid depression from non-specific stress.

Studies in pressures recorded within bone marrow cavities in dogs. GORDON F. VAWTER,* IRVING M. RASGON* AND JOHN P. MARBARGER. *Aeromed. and Physical Environment Lab., Univ. of Illinois, Chicago, Ill.*

In dogs, (9–20 kg. in weight), anesthetized with sodium pentobarbital (Nembutal), pressures within the marrow cavity of long bones (obtained through a self-sealing cannula piercing cortical bone), were compared with carotid artery pressures during various procedures following a control period. A pulse wave was recorded which was similar in contour to that obtained at the origin of the nutrient artery of the marrow. The range of systolic pressures in the marrow in over 150 determinations was 7.9–141.2 mm. Hg; the range of diastolic pressures was 5.6–122 mm. Hg, and the range of pulse pressures was 1.9–41 mm. Hg. The mean values of these pressures were 57.6, 44.1, and 11.6 mm. Hg, respectively. The pressure in the bone marrow cavity ranged from 7.05–97% of the carotid artery pressure. Roughly 26.5% of the determinations fall in 0–25% range, 44% in the 25–50% range, 22.5% in the 50–75% range and 6.5% in the 75–100% range. Various mechanical, electro physiologic and pharmacologic procedures, altering systemic pressures and cardiac output: *1*) reaffirmed previously demonstrated nervous control of bone marrow vascular dynamics, *2*) demonstrated the expected relationships between blood flow in the marrow and pressure changes, and *3*) emphasized the role of delivery pressure of blood on the pressure within the closed, fixed volume of the marrow space. Disproportionate drops in the bone marrow pressure were observed following relatively small induced hemorrhage. Various clinical and experimental applications are being explored.

Rapid, massive intravenous infusions of 'normal saline' in dogs. GORDON F. VAWTER,* C. BRUCE TAYLOR* AND JOHN P. MARBARGER. *Aeromed. and Physical Environment Lab., Univ. of Illinois and Dept. of Pathology, Presbyterian Hospital, Chicago, Ill.*

Various cardio-vascular dynamic and electrolyte shift studies were performed 16 times on 7 dogs, (15–22 kg. in weight), anesthetized with sodium pentobarbital—Nembutal) before, during, and for 8 hours following intravenous infusion. The 'normal' saline was infused at a rate of 50 cc/minute until 150 cc/kg. had been administered. Experiments were conducted on each dog before and at intervals following massive right and left ventricular freezing as described in a forthcoming issue of *Circulation*. The first stress was circulatory, (1 of 7 control dogs died during infusion). The second stress, (probably involving water and electrolyte distribution) operated several hours

after infusion. One control dog with a precordial murmur and thrill showed fluid and electrolyte retention and prolonged general vascular engorgement, with recovery in the presence of the normal pattern of cardiac output. More 'normal' results followed digitalization of this dog. Dogs with right ventricular damage (50%) showed hyperresponse in stroke volume (from lowered initial levels), cardiac output, and venous pressure with delayed recovery during which peripheral resistance rose markedly. These findings were associated with urinary fluid and electrolyte retention. The pulmonary circuit was not involved. One right ventricle dog died 4 hours after infusion 6 weeks after injury. A dog with left ventricle lesions (50%) had depressed arterial pressures and cardiac outputs which showed hyperresponse to stress. The venous pressure rises were transient and not excessive, although congestion of the pulmonary circuit was noted (cyanosis, hydrothorax, and pulmonary edema). Urinary retention of electrolyte and water tended to be less than in right ventricle dogs.

Pituitary and adrenal ascorbic acid in x-irradiated rats. K. D. VENTERS* AND E. E. PAINTER. *Univ. of Illinois, College of Medicine, Chicago.*

The purpose of this investigation was to determine whether x-radiation of the rat altered the ascorbic acid levels in the anterior pituitary and adrenal cortex. Both glands have high concentrations of ascorbic acid which is seen normally in tissues with marked oxidation-reduction activity. The adrenal, in particular, shows fluctuations in ascorbic acid with changes in secretory activity resulting either from pituitary stimulation or from ACTH injection. Sprague-Dawley male rats weighing 250–330 gm. were given 770 r total body x-radiation. Ascorbic acid levels were determined by the Roe and Kuether method at 1, 4, 18 and 72 hours after radiation. A decrease of 34% in adrenal ascorbic acid occurred during the first hour. Following this there was a gradual increase to normal levels by 18 hours. Weight changes at 18 hours indicated hypertrophy which was still increasing at 72 hours. In the pituitary there was an increase of 2.5% during the first hour, which continued to 13.5% by 72 hours after radiation. However, the pituitary weight decreases by 23.5% during this period. These results for adrenal weight changes and ascorbic acid levels after radiation followed the same pattern as those obtained by other investigators and are accounted for by pituitary stimulation. The changes in the pituitary also appear to be a function of increased cellular activity with excessive hormone production. Confirmation of this was reached by histochemical studies.

Subliminal excitation and refractoriness in single photoreceptor elements. H. G. WAGNER,* H. K. HARTLINE AND I. H. WAGMAN. *Jenkins Labs. of Biophysics, Johns Hopkins Univ., Baltimore, Md., and Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

In individual photoreceptor units of the eye of *Limulus*, subliminal illumination produces characteristic changes in excitability. These changes were measured by determining the intensity required for a 10-msec. test flash to reach threshold at various times preceeding, during or following an exposure of fixed subliminal intensity. During prolonged subliminal illumination, excitability was a maximum when the test flash was given about one second following onset; thereafter it diminished somewhat, but remained elevated above its resting level in darkness. When the light was turned off, the excitability dropped abruptly, the receptor passing into relative refractoriness from which it recovered in a few seconds. The greater the intensity of the subliminal illumination, the greater was the maximum of excitability, the higher its plateau, and the greater the refractoriness after the light was turned off. For a short subliminal flash, a similar rise and fall of excitability, followed by a few seconds of relative refractoriness, were observed, The rise of excitability could not be measured accurately, but an analysis of the time of occurrence of the response when the test flash preceded the subliminal flash showed that the rise must have been more rapid than the fall.

Comparison of circulatory effects of l-epinephrine and l-nor-epinephrine. KHALIL G. WAKIM AND HIRAM E. ESSEX. *Section on Physiology and Div. of Exptl. Medicine, Mayo Fndn., Rochester, Minn.*

The effects of l-epinephrine bitartrate and of l-nor-epinephrine bitartrate in the circulatory system were studied in heparinized dogs anesthetized with pentobarbital sodium or ether. The drugs were administered either intravenously or intraarterially. The dosage varied from 0.01–10 μg/kg. of body weight. The heart rate was recorded electrocardiographically; the blood pressure, by use of a mercury manometer connected to the carotid artery. The blood flow in the two femoral arteries and in addition sometimes in one femoral vein and one carotid artery was measured by use of bubble flow meters. The simultaneous changes in blood pressure, heart rate and blood flow produced by l-epinephrine were indistinguishable from those produced by l-nor-epinephrine. Intravenous injections of either drug in equivalent doses produced an immediate rise in blood pressure of practically the same magnitude and the resulting tachycardia was followed by reflex bradycardia during the peak of increased blood pressure. Im-

mediately before the maximal increase in arterial blood pressure after the intravenous injection of either l-epinephrine or l-nor-epinephrine, a transient increase in blood flow of several hundred % accompanied the augmentation in cardiac output. This was attributed to the direct stimulatory effect of the drugs on the heart. Often a moderate or slight reduction in blood flow occurred within about 4 minutes before the return to the pre-injection level. Intraarterial injection of l-epinephrine or l-nor-epinephrine brought about an immediate vasoconstriction in the supplied area with reduction or cessation of blood flow in the skinned as well as the intact limb. The degree and duration of the reduction or cessation of blood flow depended on the dose. Small doses caused slight and transient reduction in blood flow without significant effect on heart rate or arterial blood pressure. Large doses caused complete cessation in blood flow in the injected arteries independent of the effects on the heart rate or arterial blood pressure.

Presence of two amylases in dog's pancreatic juice. JEROME M. WALDRON (introduced by I. H. WAGMAN). *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

The relationship of the amyloclastic and saccharinogenic properties of dog's pancreatic juice was investigated by comparing the residual turbidity of a stable standard starch suspension and the amount of reducing substance produced at various times after digestion was initiated. Determinations of these two properties were made at various periods of digestion on 25 different samples of pancreatic juice from the same dog. For an individual sample of pancreatic juice these two properties are mutually dependent because the relationship of reducing substance formed to turbidity is linear. In the aggregate there was no correlation of these two properties except the expected tendency of the formation of greater amounts of reducing substance when greater digestion had occurred. The amyloclastic and saccharinogenic properties of dog's pancreatic juice, therefore, vary independently in different samples. In addition, when the pancreatic juice is refrigerated for 24 hours these two properties may be increased or decreased but not necessarily in the same direction. This dissociation of the amyloclastic and saccharinogenic properties of dog's pancreatic juice suggests that there are two amylases present and that they are not secreted in parallel.

Similarity of the double response and of tetanus in frog and rat skeletal muscle at similar temperatures. SHEPPARD M. WALKER. *Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky*

The amount of summation of tension resulting from indirect repetitive stimulation of the frog gastrocnemius at room temperature (20°C.) is smaller than the amount resulting from indirect repetitive stimulation of the rat triceps surae at body temperature (37°C.). The possibility that the difference between room temperature and mammalian body temperature may account for the observed difference between tetanic response in frog muscle and tetanic response in rat muscle, suggested itself. When studies were carried out at similar temperatures, it was found that the responses of frog and rat muscle are similar. At 35°C. the ratio of double response tension to twitch tension is about 2.2, and the ratio of tetanic tension to twitch tension is about 5 in both animals. At 20°C. the ratio of double response tension to twitch tension is less than 2, and the ratio of tetanic tension is about 3. Reduction of muscle temperature from 35°C. to 20°C. decreases tetanic tension 20% in the frog and 25% in the rat. The time course of the twitch response is markedly increased by cooling. It is suggested that the reduction of tetanic tension, induced by cooling, is dependent upon the accompanying reduction in speed of single responses. It is also suggested that the difference between the amount of summation of twitch tension in rat muscle at body temperature and the amount of summation of twitch tension in the frog muscle at room temperature is due, at least in part, to the difference in temperature.

Some actions of strychnine. PATRICK D. WALL,* JEROME Y. LETTVIN,* WARREN S. MCCULLOCH and WALTER PITTS.* *Univ. of Chicago, Chicago, Ill.; Manteno State Hosp., Manteno, Ill.; Dept. of Psychiatry, Univ. of Illinois College of Medicine, Chicago; and Massachusetts Inst. of Technology, Cambridge.*

Strychnine was found to increase the height of the action potential of dorsal root fibers of cat spinal cord and to occlude potentiation by diminishing the time constant. On the other hand, strychnine did not consistently or markedly increase antidromic response. It is proposed tentatively that strychnine acts by affecting mainly presynaptic elements in the same direction as potentiation.

Mechanism of interstitial protein mobilization by saline infusions. KARLMAN WASSERMAN* AND H. S. MAYERSON. *Dept. of Physiology, School of Medicine, Tulane Univ., New Orleans, La.*

Dogs anesthetized with Pentothal are infused with volumes of physiological saline solution amounting to 1–3 times their plasma volumes at rates varying from 10–50 ml. per minute. Judged by changes in the rates of disappearance of intravenously injected radioactive iodo-albumin, these

infusions increase the transudation of albumin from the circulation as much as 5½ times. Concomitantly there is an increased rate of albumin return via lymph which is approximately three times as great as that of the pre-infusion period. This increased albumin return masks the true loss of albumin from the plasma, and in many cases, actually exceeds the loss and results in increased (20%) levels of total circulating albumin. If the lymph is not returned to the circulation or if the lymph is replaced by saline, the total circulating plasma protein decreases by 15–30% in 3 hours. Specific activity data indicate that no new albumin is involved in the process but that the infusion serves to mobilize interstitial protein for return via lymph to the blood stream. The data suggests that the infusion results in the transudation of increased amounts of dilute plasma filtrate from the circulation. This then mixes with the interstitial fluid containing a higher concentration of albumin than the plasma filtrate and results in lymph of relatively high albumin concentration. The level of total circulating plasma albumin is thus a function of the availability of interstitial albumin and of lymph flow and the ability of this process to compensate for the leakage of albumin from the plasma.

Clotting time and interaction of bovine fibrinogen and thrombin. DAVID F. WAUGH AND BETTY J. LIVINGSTONE.* *Dept. of Biol. Massachusetts Inst. of Technology, Cambridge.*

At pH 6.85, ionic strength 0.15 and 22.7°C. activated fibrinogen appears according to:

$$-\frac{d\phi}{dt} = \frac{0.482\,\mathrm{Th}_0}{0.051 + \phi_0}\,\phi \qquad (1)$$

Initial fibrinogen, ϕ_0, and ϕ are in mg. C.N. per ml., total thrombin, Th_0, is in N.I.H. units per ml., and t in minutes. Integrating (*1*) and substituting experimental clotting times, T_c, yields:

$$\phi^1 = \phi^0 + 0.11/3.8 = \mathrm{LnTh}_c \qquad (2)$$

ϕ^1 is total fibrin, $\phi_0 - \phi$. Combined (*1*) and (*2*) give:

$$T_c = \frac{0.051 + \phi_0}{0.482\,\mathrm{Th}_0}\,\mathrm{Ln}\,\frac{\phi_0(3.8 - \mathrm{LnTh}_0)}{\phi_0(2.8 - \mathrm{LnTh}_0) - 0.11} \qquad (3)$$

According to (*1*), the rate of appearance of activated fibrinogen increases with increasing Th_0 and ϕ_0. T_c decreases with increasing Th_0 but increases with increasing ϕ_0 (above the minimum in the $T_c - \phi_0$ curves). ϕ^1 always increases with increasing initial rate (eq.'s *1* and *2*). At the time when the solution gels or when the first fibrin strands are visible (end point used) only a part of the total fibrin has been incorporated into the clot structure (clot fibrin). The remainder, potential fibrin, exists as unattached fibrin strands or activated fibrinogen. The ratio, potential fibrin over clot fibrin, increases with increasing rate. When ϕ_0 is increased, the decreased utilization of potential fibrin overcomes the increased rate of appearance of activated fibrinogen and T_c increases. When Th_0 is increased the opposite is the case. Materials, such as gum acacia, which decrease the clotting time, may not increase the rate of the reaction, but may alter substantially the utilization of potential fibrin.

Influence of hepatic blood flow upon resistance to prolonged arterial hypotension. HOWARD WAYNE,* J. T. JOYNER, JR.,* WILLIAM MCCALL* AND HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.*

The recipient (hypotensive) dog was allowed to bleed into a Lamson reservoir until its mean arterial pressure had fallen to 40 mm. Hg; the reservoir was then transferred to and equilibrated with the arterial pressure of the donor dog. Crosscirculation was then begun with the arterial blood of the donor flowing into the splenic vein of the recipient and returning from an artery of the recipient to a systemic vein of the donor. The flow in each direction was automatically adjusted by electromagnetic clamps so that the arterial pressure of the recipient remained at 37–43 mm. Hg; any change in effective blood volume in this dog was thus transferred to the donor and was in turn reflected by a change in the volume of blood in the Lamson reservoir. The crosscirculation was continued until the volume of blood in the reservoir had decreased 50%; the original volume withdrawn was then given back to the recipient dog and the animal then followed until death. In control experiments blood from the donor flowed into a systemic vein of the recipient. *Results:* Seven controls: crosstranfusion lasted 2–3.6 hours (av. 2.9 hours); survived 2.0–5.3 hours (av. 3.5 hours). Seven liver perfused dogs: crosstransfusion lasted 2–14 hours (av. 7.6 hours): survived 9–28 hours (av. 13.1 hours). One animal from each group remained alive. *Conclusions:* Maintenance of an adequate hepatic circulation greatly increases the period of arterial hypotension necessary to induce irreversible shock. (Supported by grants from the A.M.A. and the U.S.P.H.)

Acute hemodynamic effects of coronary artery occlusion. RENÉ WÉGRIA, CHARLES W. FRANK,* GEORGE MISRAHY,* HSEUH HWA WANG,* ROBERT CASE* AND ROBERT MILLER.* *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Presbyterian Hosp., New York City.*

Dogs were anesthetized with chloralose and,

under artificial respiration, the chest was opened. A ligature was placed around the left anterior descending coronary artery at its origin to produce a large infarct or in its middle third to cause a small infarction. Continuous records of cardiac output and mean arterial blood pressure were obtained with a rotameter and an optical manometer, respectively. In 6 dogs, small infarctions produced only a minimal decrease of blood pressure and cardiac output, and one minute after the occlusion, pressure and output had either stabilized or begun to return toward control. With large infarctions, ventricular fibrillation occurred in all 13 dogs, 1½–16 minutes after occlusion. The initial drop in blood pressure was similar to that seen in small infarct but the fall in cardiac output was more pronounced, indicating a rise in total peripheral resistance. In 8 dogs, after this intial phase of 1–2 minutes, blood pressure and cardiac output remained steady or fell gradually. In the remaining 5 dogs, pressure and output rose to or toward control levels with a progressive increase in the peripheral resistance.

Calcium binding in muscle. VIRGINIA WEIMAR (introduced by L. V. HEILBRUNN). *Dept. of Zoology, Univ. of Pennsylvania, Philadelphia.*

Frog muscle brei from muscle blended in Ringer's solution (1 gm. muscle to 3 cc. Ringer) took up 90% of the Ca from the Ringer. The maximum uptake of Ca by the brei was 0.124 mg. Ca/gm. of muscle. If the brei was made in Ca-free frog Ringer and if at various time intervals the Ca was added to the brei standing at room temperature, the binding power of the brei fell from 80% uptake of Ca to 40% uptake within 15 minutes. If the brei was kept on ice, it retained the ability to take up 80% of the Ca for 2 hours or more. At pH's above 5.5 the Ca uptake by the brei stayed at a constant high level. At a pH of about 5.3 the Ca uptake by the brei began to drop sharply from 85% uptake to 20% uptake at pH 4.8. Brei exposed to 37°C. for 10 minutes irreversibly lost its power to take up Ca. Only 20% was bound at this temperature, and no rebinding occurred when the brei was cooled to room temperature (23°–24°C.). At 35°C. 50% of the Ca already bound was released but was rebound when the brei was cooled to room temperature. Ca was also given up by the brei at −10°C. and rebound on warming to 0°C. Ca was released from the breis by physiological concentrations of NaCl, KCl, and Ca-free Ringer's solution. K was more effective than Na in releasing Ca.

Shortening of frog muscle fibers by serum of DASF guinea pigs. VIRGINIA WEIMER* AND ROSALIND WULZEN. *Oregon State College, Corvallis.*

The shortening rates of single muscle fibers of the frog in normal guinea pig blood serum were determined. These values were compared with the rates in the serum of guinea pigs which had developed a muscular stiffness due to deficiency of the anti-stiffness factor (DASF). Fibers were suspended by hanging-drop method in calcium-free mammalian Ringer-Tyrode. Camera lucida drawings were made at the beginning and end of a 5-minute control period. The Ringer was then replaced with serum and the exact time noted. Five minutes later a final drawing was made and the amount of shortening determined. The shortening rates in mammalian Ringer were subtracted from the rates in serum for the calculations below. The average shortening rates were 0.471 mm/minute for 14 severely stiff animals, 0.229 mm/minute for 9 moderately stiff animals, and 0.089 mm/minute for 7 normal stock animals. Data were analyzed with the Student's t test. The t values and degrees of freedom were, respectively, severely stiff vs. stock, 5.44 and 20; severely stiff vs. moderately stiff, 3.52 and 21; moderately stiff vs. stock, 2.88 and 15. These t values indicate highly significantly different rates between normal animals and animals deficient in the anti-stiffness factor and between deficient animals having different degrees of stiffness. Thus the serum of DASF guinea pigs contains muscle contracting substances.

Modification of local temperature response in an inflammatory process. H. G. WEINSTEIN (introduced by G. UNGAR). *Rheumatic Fever Research Inst., Northwestern Univ., Chicago, Ill.*

A local inflammatory process was induced in the skin of the guinea pig ear by the topical application of xylene. Vascular response and fluid exchange were studied in terms of thermometry data obtained from the surface of the ear. All temperature measurements were made in a closed, thermoregulated chamber by means of a trimetal thermo-couple circuit. It was found that the local temperature rise concomitant with the xylene inflammation in otherwise normal guinea pigs was on the average of 1.4°C. (Δt); that is $\Delta t = t_x - t_0$ The increased surface temperature accompanying the xylene induced erythema was significantly inhibited in those animals pretreated with ACTH (7 mg/kg. − $\Delta t = 0.7$), cortisone (5 mg/kg/day − $\Delta t = 0.3$), and sodium salicylate (250 mg/kg. − $\Delta t = 0.5$). Alteration of the surface temperature regulating processes in the pre-inflammatory ears of animals treated with cortisone or sodium salicylate was studied by analyzing thermal circulation in the ear with Newton's heat-transfer equations after Burton. A substance found in spleen (splenin A) also acted upon the local inflammation temperature rise; 25 μg. splenin A/kg. produced a Δt of 0.8. Factors involved in thermal

circulation as well as the anti-inflammatory action of the pituitary-adrenocortex-spleen cycle will be discussed in the light of the data presented.

Electrical activity of a simple ganglion. J. H. WELSH AND D. M. MAYNARD, JR.* *Harvard Univ., Cambridge, Mass.*

The cardiac ganglion of the lobster, *Homarus americanus*, contains five large motor neurons and four small neurons. The ganglion may be exposed by a ventral approach through the floor of the heart and the electrical activity of the component cells recorded while the heart continues to beat, or the entire ganglion, or parts of it, may be isolated and studied. In the semi-intact heart which is beating normally a burst of nerve impulses (series of discharges from several cells) precedes and accompanies the early part of the mechanical response. The pattern of impulses may be remarkably constant throughout a long series of contractions. When the isolated ganglion is placed on recording electrodes the discharges of the large cells may occur in bursts or, after a period of time, one or more cells may discharge with a regular rhythm. Each cell may have an independent rhythm, or, at times, there is an obvious interaction between neurons with two or more cells firing in rapid sequence. The repetitive discharge of a single cell in an entire ganglion, or segment of ganglion, without obvious activity of other cells would appear to be an example of 'spontaneous' activity. Acetylcholine applied to a semi-intact ganglion decreases the interval between bursts thus increasing the frequency of heart beat; when applied to the isolated ganglion in which cells are firing repetitively the rate of discharge is increased. The increase may be followed by slowing and block if the concentration of acetylcholine is high. Epinephrine decreases the frequency of firing.

Evaluation of hepatic blood flow in the dog. ATTIE YVONNE WERNER* AND STEVEN M. HORVATH. *Dept. of Physiology, State Univ. of Iowa, Iowa City.*

The hepatic blood flow in dogs has been estimated by measuring the hepatic extraction of bromsulphalein. The two rates of infusion were controlled in order to maintain a peripheral BSP level of less than 1 mg. % at low infusions and greater than 1 mg. % but less than 4 mg. % at higher infusions. Sherlock (*J. Lab. & Clin. Med.* 35: 923, 1950) has reported data on man indicating significantly higher calculated hepatic blood flows when the peripheral BSP level was less than 1 mg. %. No such differences were observed in the present experiments. The hepatic blood flow in dogs, when calculated by and utilizing the precautions of the method suggested by Bradley (*J. Clin. Investigation* 24: 890, 1945) showed a total variation averaging 25% during the 80-minute infusion period. The difference of calculated flows between any 10-minute sampling period averaged 13%. The relationship of the BSP technique to other procedures of determining hepatic blood flow will be discussed.

Relationship between fitness for strenuous work and ability to withstand high headward acceleration. JANET WESSEL* AND LAURENCE E. MOREHOUSE. *Univ. of Southern California, Los Angeles, and The Air Force School of Aviation Medicine, Randolph Field, Texas.*

Each subject was exposed to $+5g$ until he greyed out or until he had endured $+5g$ for 30 seconds. Fitness for strenuous work and g-tolerance measures were taken before the training program began, at the end of the training program, and 8 weeks after the training program was stopped. The basic exercises used in the 6½ weeks' training program were weight training and running which gave improved scores in tests of muscular strength and cardiovascular endurance. Improvement in physical fitness scores was invariably accompanied by improved g-tolerance. Cessation of frequent and regular exercise was accompanied by decreased g-tolerance. The relationship between fitness for strenuous work and g-tolerance at any given time was positive but only slight, demonstrating that muscular strength and cardiovascular endurance are factors in g-tolerance but not the only important ones.

Relationship of calcium and magnesium to effects of phenobarbital on Q_{O_2} of brain slices. B. A. WESTFALL. *Dept. of Physiology and Pharmacology, Univ. of Missouri Med. School, Columbia.*

The effects of calcium and magnesium on the oxygen consumption of rat cerebral cortex slices in the presence of phenobarbital have been studied. The direct method of Warburg was used in which the slices respired in Kreb's-Ringer's-Phosphate-Buffer solution (varying in Ca or Mg content) carrying 0.2% glucose. Two series of experiments were conducted, with 10 animals at every point. Results given are statistically significant. In *series 1*, 0.3 mg.% phenobarbital tipped in (no calcium) after a 30-minute control period produced a decrease of 6%, however, when the medium contained either 0.11 M or 0.22 M calcium the barbiturate caused a 17% increase in oxygen consumption. Twenty mg.% phenobarbital decreased the oxygen consumption 22, 16 and 13% in media with no calcium, 0.11 M calcium and 0.22 M calcium respectively. *Series 2* was a duplicate of *series 1* except that calcium was maintained and the magnesium content was varied. With no magnesium present 0.3 mg.% phenobarbital had no effect on oxygen consumption, but in 0.154 M

and 0.308 M magnesium the oxygen consumption was increased 9.6 and 5.5% respectively. Twenty mg.% phenobarbital produced a decrease in oxygen consumption of 14, 12 and 14% in solutions containing no magnesium, 0.154 M and 0.308 M respectively. The data indicate that both calcium and magnesium are necessary for proper amounts of phenobarbital to produce an increase in oxygen consumption of brain slices, but neither calcium nor magnesium in the concentrations used seems to affect the degree of depression of oxygen consumption caused by the barbituarte.

Significance of capacitance changes accompanying cardiac activity in man. WILLIAM V. WHITEHORN. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Further study of capacitative changes accompanying cardiac activity (cardiodielectrograms) has provided evidence regarding significance of such changes. Theoretical considerations and experimental evidence indicate that primary factors in production of cardiodielectrograms are volumetric and mechanical changes in the thorax secondary to cardiac activity, rather than direct effects of changes in dielectric content of the heart. Similarity of capacitance curves to ventricular electrokymograms suggests the importance of ventricular events in their production. Observations on normal subjects have established characteristic patterns, and specific points on cardiodielectrograms are readily identified with phases of the cardiac cycle. Figures for duration of these phases in normals show good agreement with accepted values. Distortion of normal pattern may occur with cardiac disease. Magnitude of capacitance change is related to stroke volume. In 132 determinations on 20 subjects simultaneous estimations of relative changes in stroke volume with low frequency ballistocardiograph and dielectrocardiograph showed agreement within 20% in 110 cases (83.5%) and within 25% in 117 (88.5%). The method invites comparison with ballistocardiography and electrokymography as an empirical adjunct to study of human cardiac activity.

Disposal of glucose by extra-hepatic tissues under influence of insulin. ARNE N. WICK* AND DOUGLAS R. DRURY. *Scripps Metabolic Clinic, La Jolla, Calif. and Dept. of Physiology, Univ. of Southern California, Los Angeles.*

It has been shown by the use of C^{14}-labeled glucose in eviscerated rabbits that insulin increases the oxidation of glucose to CO_2 by the extra-hepatic tissues (*J. Biol. Chem.* 188: Jan., 1951). Equilibrium between the glucose disappearance from the blood and the expired CO_2 is not reached before 8–10 hours. Without insulin, equilibrium is attained in 3–4 hours. This indicates, particularly in the insulinized animal, the presence of a large pool of glucose intermediates in process of metabolism. Examination of the carcass reveals that the glucose which is not oxidized is disposed of chiefly as water-soluble compounds with lesser amounts in glycogen and protein-like material. Only a small fraction of the water-soluble fraction is 'free glucose' and the amount of this is not increased in insulinized animals over controls. The results obtained by the fractionation of the water soluble fraction will be reported.

Inconsistency of vascular responses to low concentrations of epinephrine topically applied to the rat mesentery. MARY P. WIEDEMAN* AND PAUL A. NICOLL. *Dept. of Physiology, Indiana Univ., Bloomington.*

Using the test site and method developed by Zweifach *et al.* with the exposed cecal mesentery of the anesthetized rat, a study of the stability of the response of small blood vessels to topically applied epinephrine was carried out. The results of each test are reported in terms of the lowest concentration that produces a detectable change of flow in the observed vessels. Reproducibility of a determined threshold concentration, using both powdered levo-epinephrine and commercial epinephrine (Parke, Davis & Company) was considered as demonstrated if three applications gave a response while alternate tests with the next lower concentration were negative. Less than 50% of all tests fulfilled this empirical criterion and many showed wide fluctuations in threshold during a test series. In the attempt to stabilize threshold values and increase reproducibility, several technical and procedural modifications were tried. No correlation between stability and temperature of the drip solution or its gelatin concentration was found. Changes in the concentration of the applied epinephrine due to auto-oxidation were ruled out. Ephedrine pretreatment, while tending to lower epinephrine thresholds, did not stabilize these threshold values. Ferritin injections also gave no consistent shift in epinephrine thresholds nor stabilized the apparent values. A positive correlation between the depth of anesthesia and responsiveness to epinephrine was apparent. These data add further caution to interpretations of changes in adrenaline thresholds as supporting, or deriving from, fundamental physiological processes.

Experiments on the microinjection of adenosine triphosphate into skeletal muscle fibers. FLOYD J. WIERCINSKI AND B. A. COOKSON.* *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

The response of muscle fibers to microinjection

of ATP is dependant on salt concentration. At low Na^+ concentration contraction takes place. At high Na^+ concentration it is inhibited. This is interpreted as meaning that the actomyosin of resting muscle is dissociated and hence will not contract in the presence of free ATP. 0.05 M phosphate (pH 7.0) blocks the action of 0.003 M ATP over a range of Na^+ concentration at which if chloride alone were present contraction would occur. It is suspected from this that the anions as well as the cations of muscle play a part in keeping actin and myosin dissociated while the muscle is at rest. Osmotic effects are considered not to interfere greatly with the interpretation of results of clean microinjection experiments, but in unclean microinjection experiments where the outside of the fiber is contaminated with the injectant, osmotic effects materially modified the results.

Effect of psycho-motor stress on blood pressure of normal dogs. C. M. WILHELMJ, T. MCGUIRE* AND E. B. WALDMANN.* *Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Nebr.*

Stress was produced by confinement from 4–18 hours in a cage treadmill revolving between 1 and 2 mph. The number of sessions per week varied from 1–7. Even after the longest sessions the animals did not appear unduly fatigued, but most of them came to dislike the procedure. Blood pressure was determined daily by the method of Allen, 5–6 hours after removal from the treadmill. Seven dogs have been studied. Four showed significant and marked elevation of pressure, systolic more than diastolic. One dog showed little elevation and 2 others only slightly more. There is often evidence of a cumulative effect in that the first few sessions cause little or no elevation but repeated sessions gradually elevate the pressure. On several occasions repeated sessions caused marked elevation of pressure which required a week or more to return to normal. One dog was given several 18-hour sessions per week for 4 months and the pressure was nearly constantly elevated but in this as in most other dogs the pressure returned to normal after several days rest. There was no definite evidence of adaptation. In the animal run for 4 months sudden cessation of the stress caused a marked and significant drop in pressure below the control level which persisted for several weeks. High salt intake did not appear to alter the response.

Regional vasomotor tone in normotensive and hypertensive dogs. ARNOLD H. WILLIAMS* AND HENRY A. SCHROEDER. *Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.*

The asystolic arterial pressure gradient, defined as the descending curve of intra-arterial pressure following sudden occlusion of a major artery, can be used as a measure of regional peripheral resistance (WILLIAMS, A. H. AND H. A. SCHROEDER. *Am. J. Physiol.* 155: 132, 1948). That function of the gradient which is most applicable in measuring vasomotor tone is the level of intra-arterial pressure 6 seconds after occlusion or EP_6. It is but little affected by levels of arterial pressure or rates of flow during the determination of brachial and renal pressure-flow curves. By the combined use of these curves and gradients an arbitrary scale was set up which provides a means for estimating the actual degree of constriction. Levels of EP_6 are affected by collateral circulation but the degree of the latter can be considered constant in large series such as were used. Values of EP_6 were determined in brachial, femoral, renal, and mesenteric beds to evaluate the pattern of regional vasomotor tone of anesthetized normotensive, renal and neurogenic hypertensive dogs. The correlation of diastolic pressure with EP_6 was best in the mesenteric circulation, poorest in the kidney. The production of acute neurogenic hypertension causes a uniform vasoconstriction. Both chronic neurogenic and chronic renal hypertension were accompanied by generalized vasoconstriction, most prominent in the mesenteric area. It was less marked in the limbs of the neurogenic group. The results obtained in the renal hypertensive kidney may indicate that intrarenal resistance is markedly increased.

A continuously recording Geiger counter scanning device for radio chromatographic analysis of labelled antigen-antibody reactions. ROBERT R. WILLIAMS AND ROBERT E. SMITH (introduced by ORR E. REYNOLDS). *Natl. Insts. of Health, Bethesda, Md.*

An evaluation is presented of a continuously recording counter device which has been adapted to the scanning of radio chromatograms. This method has been applied to a chromatographic method of demonstrating and measuring the reaction between I^{131} labelled bovine serum albumin and its antiserum. Substantial agreement has been obtained between the maximum precipitation zone found by this method and that obtained by a standard method. The method permits quantitative measurement of precipitin reactions with one-hundredth of the quantity of antiserum required by the usual methods. Further it has been possible to demonstrate soluble antigen-antibody complexes in the region of antigen excess and has facilitated the study of other protein-protein complexes under conditions in which all components are soluble. Studies are in progress which

promise to provide some theoretical treatment of the chromatography of proteins.

Interaction on blood pressure of ergonovine and epinephrine. J. H. WILLS. *Pharmacology Section, Med. Div., Army Chemical Center, Md.*

Ergonovine maleate in small doses (0.5–1.0 mg/kg.) has been found to enhance the pressor effect of epinephrine (5 μg/kg.). In larger doses (2.5 mg./kg) ergonovine maleate decreases the pressor effect of epinephrine. Because both epinephrine and ergonovine possess secondary amino and alcoholic hydroxyl groups separated by two carbon atoms, it is suggested that this is another example of synergy between small doses of derivatives of ethyl amine and competition between larger doses (C.-S. JANG. *J. Pharmacol. & Exper. Therap.* 70: 347, 1940).

Changes in the protoplasmic cortex during cell division. WALTER L. WILSON (introduced by F. J. M. SICHEL). *Dept. of Physiology and Biophysics, Univ. of Vermont College of Medicine, Burlington.*

A number of biologists have emphasized the importance of the cell cortex in various cell processes. And certainly there is little doubt that the protoplasmic cortex does play a significant role in the life of a cell. During the course of cell division changes occur in the colloidal state of the cell cortex. In the egg of the annelid worm, *Chaetopterus pergamentaceus*, granules can move from the cortex under moderate centrifugal force, and the force necessary to dislodge the granules varies during the mitotic cycle. In the present work, eggs were subjected to various centrifugal forces at intervals between fertilization and cleavage and the cell cortex examined under high power magnification to determine whether or not the granules were present in the cortex. All experiments were carried out at a temperature of 21° C. Following fertilization the rigidity of the cortex progressively decreases for about 5 minutes. Then the rigidity increases again and at about 10 minutes after fertilization the cortex has returned to its original state. The cortex remains in this condition until about 35 minutes after fertilization at which time there is again a decrease in rigidity. From about 40 minutes after fertilization until cleavage there is no further change in the colloidal state of the cortex. Experiments carried out while the cleavage furrow was forming show that there is no increase in the rigidity of the cortex at this time.

Optimal time for change of bath fluid in artificial kidney. A. V. WOLF, JOHN E. KILEY* AND GORDON D. CURRIE.* *Dept. of Physiology and Pharmacology and of Medicine, Albany Med. College, Albany, N. Y.*

In order to augment the exchange of hemodialyzable solutes in a Kolff-type artificial kidney during a total operating time, t, the bath may be drained and refilled with fresh fluid at an intermediate time, t_i. Where dialytic blood flow is kept constant, the removal of urea from blood to bath follows closely an exponential curve. It is shown graphically and by general mathematical solution that the maximal removal of urea (ignoring its rate of formation) with a single change of bath fluid occurs when $t_i = t/2$. All operating time intervals, t'_i, between n changes should therefore be equal, and $t'_i = t/(n + 1)$. For a total operating time, $t = 6$ hours, it may be found that with one bath change at 3 hours, the urea removal from the body is ca. 16% above that which would have occurred with no bath change. With two changes (2 and 4 hours) the increment is ca. 20%. With an infinite number of changes the calculated increment is ca. 31%. These particular approximations are for a system having a bath volume of 100 liters, a 70-kg. man, and a dialytic urea clearance at zero time of 175 cc/min.

Effect of thiouracil induced hypothyroidism on the partition of radioactive calcium between bone and liver in normal and rachitic male rats. L. F. WOLTERINK, D. I. EPSTEIN AND M. MURRAY (introduced by W. D. COLLINGS). *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Thiouracil injection (10 mg/rat/day for 2 weeks) into young male Rockland rats weighing 90 grams on a normal ration reduced the turnover of subcutaneously injected Calcium 45 in liver. Despite greater retention in this soft tissue, retention of radio-calcium in the femurs of the hypothyroid animals was reduced although the total femur calcium was not significantly altered. In terms of calcium space, hypothyroidism increased the apparent calcium space in the liver but decreased the apparent calcium space in the femur of animals on a normal ration. The total retention in these two spaces was decreased 42% by thiouracil. Apparently a smaller proportion of the total femur calcium is in exchange equilibrium with injected Calcium 45 in the hypothyroid animal on a normal ration. The same experiment repeated on rachitic rats wieghing 50 grams showed the characteristic inability of the vitamin D deficient controls to retain injected Calcium 45 in the femur pool. The reduced apparent pool size allowed much more rapid turnover in the rachitic controls than in the normal controls. Thiouracil induced hypothyroidism partially protected the femur pool against the vitamin D deficiency. The apparent calcium pool of the liver was increased, as noted in the experiment with a normal ration. The total retention in liver plus femur

was probably increased by thiouracil in the rachitic rats due to the predominant effect of the goitrogen in protecting the bone pool against the avitaminosis. (This work was performed under contract with the U. S. Atomic Energy Commission at Michigan State College, Esat Lansing.)

Effect of variation in size of shock dose of antigen in canine anaphylaxis. PARKE H. WOODARD (introduced by O. O. STOLAND). *Dept. of Physiology, Univ. of Kansas, Lawrence.*

Dogs were actively sensitized to horse serum. At varying periods of time following such sensitization of other anesthetized dogs. Arrangements were made to take blood pressure records on both actively and passively sensitized animals and then they were given repeated doses of antigen in sizes much less than the 1 cc per kg. body weight which has been previously used. It was found, contrary to the experience of some, that while the sensitized dogs would shock to a small dose of antigen, that they usually recovered rapidly and that some of them were not desensitized by the reaction but would shock again to subsequent injections given shortly after the first. These reactions do not seem to be connected with the so-called circulating antibody since they are not paralleled by the ability to passively sensitize normal animals. The results were as follows: 22 animals were proven actively sensitized. Of this number all but 10 were desensitized by the first shock dose of antigen. Seven of these latter 10 were capable of passively sensitizing other animals but 3 were not. However 6 other dogs were capable of passive sensitization but did not react to additional doses of antigen. Only 3 of 17 passively sensitized dogs responded a second time to antigen injection.

Extrarenal actions of desoxycorticosterone (DCA) and adrenocorticotrophic hormone (ACTH) on tissue electrolytes. DIXON M. WOODBURY (introduced by GEORGE SAYERS). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

Bilaterally nephrectomized rats injected with either DCA (10 mg.) or DCA (10 mg.) plus ACTH (1 mg. every 4 hours) were sacrificed 24 hours after nephrectomy and samples of plasma, muscle, brain, liver, bone and gastrointestinal tract removed for analysis of water, sodium, potassium and chloride. Data on muscle electrolytes are presented in the table. Data on other tissues will be discussed. Nephrectomy was followed by a significant reduction in the concentration of muscle sodium. DCA prevented this decrease. The combination of DCA and ACTH induced a marked loss of both sodium and potassium from muscle; sodium space was actually less than chloride space in these animals. It is apparent that ACTH-induced secretion of the adrenal cortex and DCA markedly influence tissue electrolytes in the absence of the kidneys.

	INTACT UNTREATED	NEPHREX UNTREATED	NEPHREX DCA	NEPHREX DCA + ACTH
H_2O, %	77.3±0.1	78.4±0.7	77.3±0.2	77.6±0.1
Na space, %	15.19	13.21	15.15	10.98
Cl space, %	11.84	12.52	12.64	12.49
Na^1	22.7±0.5	19.9±0.5	23.1±0.7	16.2±0.7
K^1	114±0.4	116±2.1	113±1.0	100±3.2
Cl^1	15.1±0.3	14.6±0.3	15.3±0.8	14.4±0.2

[1] mM/kg. fat-free wet muscle.

Spontaneous electrical activity in the developing frog embryo. V. J. WULFF, S. M. DANCOFF,* HENRY QUASTLER* AND S. MERYL ROSE.* *Univ. of Illinois, Chicago.*

Developing frog embryos exhibit a spontaneous electrical activity during the period just preceding the appearance of the dorsal lip. This electrical activity consists of transient spikes of about 0.1-second duration which appear rather periodically for short intervals of time, i.e. 2–10 minutes. The electrical signals seem to be localized in the region of the gray crescent; they first appear 3–4 hours before the onset of gastrulation and exhibit pain but not absolute regularity. This investigation was stimulated by theoretical considerations pertaining to the problem of the embryonic organizer and differentiation. It is possible that the spontaneous electrical signals may be associated with organizer activity.

Lower incisor length in DASF guinea pigs. ROSALIND WULZEN, HUGO KRUEGER AND ALICE PLYMPTON.* *Oregon State College, Corvallis.*

Abnormally long lower incisors, among other dental irregularities, are often noted in guinea pigs maintained on diets deficient in the anti-stiffness factor (DASF). The average length of lower incisors in stock animals 10–17 months old was 12.1 mm. for 30 males and 10.4 mm. for 14 females. The length in DASF animals of same age span was 13.0 mm. for 36 males and 12.0 mm. for 27 females. In some 10–17 month DASF guinea pigs of both sexes and in many older DASF animals a rapid increase in incisor length developed, and yielded lower incisors reaching 17–20 mm., whereas no stock male had lower incisors exceeding 15.2 mm. and female 11.7 mm. To study rates of growth, the lower incisors were notched about 3 mm. above the gum line by a one-half inch carborundum dental disk attached to an electric

hand drill. The distance of the notch to the pink gum line was measured once weekly using vernier calipers. Tooth growth caused the notch to reach the distal end of the incisor and be worn off within 3 weeks in 16 out of 51 animals and within 4 weeks for the remainder. The average weekly rate of lower incisor growth was 2.2 mm. for 15 stock males and 2.1 mm. for 4 stock females. The average weekly rate of growth for 19 DASF males was 1.9 mm. and for 17 DASF females was 1.8 mm. Thus, increased length of lower incisors in DASF guinea pigs is due, not to increase in rate of growth, but to some other cause, possibly a decrease in grinding ability.

Conversion of C^{14} labeled acetate and cholesterol to adrenocortical hormones by perfused adrenal glands. A. ZAFFARONI,* O. HECHTER AND G. PINCUS. *Dept. of Biochemistry, Univ. of Rochester Med. School, Rochester, N. Y. and Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.*

In previous work we have established that isolated cattle adrenals perfused with homologous blood containing ACTH synthesize and release a mixture of corticosteroids, the principal components of which are 17-hydroxycorticosterone and corticosterone (HECHTER *et al. Recent Progr. Hormone Research.* In press). In this study, glands were perfused with blood containing ACTH plus C^{14} carboxyl-labeled acetate or C^{14} cholesterol, labeled at carbon atom 3 of the steroid nucleus, prepared by Dr. Erwin Schwenk from labeled cholesterone. After recirculating the perfusion medium through a series of 5 glands for 4 hours, the corticosteroids present in the perfusates were extracted with charcoal (HECHTER *et al. Arch. Biochem.* 25: 459, 1950) and fractionated by paper chromatography (ZAFFARONI *et al. Science* 111: 6, 1950). Following perfusion with either labeled acetate or cholesterol, both the 17 hydroxycorticosterone and corticosterone isolated from perfusates had approximately the same specific activity, which varied from 26 to 340 c/m/m (corrected for background) in the various experiments, depending upon the perfusion conditions. The radioactivity of the isolated corticosteroids was not due to a radioactive contaminant since the specific activity was not decreased by either rechromatography of the free steroids or by formation of the steroid acetates and propionates (the steroid esters were likewise obtained in pure form by paper chromatography). These observations demonstrate definitely that cholesterol can be transformed by adrenal enzyme systems to 17 hydroxycorticosterone and corticosterone. Whether cholesterol is an obligatory intermediary in the synthesis of corticosteroid hormones from acetate, remains for future investigation.

Possible sources of relaxin in the rabbit. M. X. ZARROW. *Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.*

It has been shown that the ovary is the principal source of relaxin in the pregnant sow. In this animal the hormone may reach a concentration of 10,000 G.P.U./gm. of fresh ovary during late pregnancy. In the rabbit, however, physiological data would suggest that relaxin may be produced at other sites in addition to the ovary. Assays for relaxin were carried out on various tissues of the rabbit to determine the possible sites of production of the hormone. Fresh tissue was ground up in 3% HCl and extracted in the cold. Four % NaCl was added and the supernatant adjusted to pH 7 and dialyzed. Various dilutions were then tested in castrated guinea pigs primed with estradiol. In the pregnant rabbit the uterus showed a relaxin concentration of 10 G.P.U./gm. of fresh tissue, the placenta 50–75 G.P.U./gm. and the ovaries 25–30 G.P.U./gm. Castration at midterm and maintenance with progesterone resulted in normal relaxin concentration in the blood when the placentae were maintained indicating that the ovary was not the major source of relaxin in the rabbit during pregnancy. In several instances where the relaxin of the blood fell to 5 G.P.U./ml., partial resorption of placentae was noted. Injection of chorionic gonadotropin into normal, estrous rabbits resulted in a concentration of 0.3 G.P.U./ml. blood serum, 5 G.P.U./gm. of ovary, and 50 G.P.U./gm. of uterus. It would thus appear that relaxin may be produced either in the ovary, uterus, or placenta and that the latter two are the main sites during pregnancy. Chronic treatment of castrated, hysterectomized rabbits with 50 μg. of estradiol daily resulted in a level of 0.2 G.P.U./ml. of blood and 5 G.P.U./gm. of vaginal tissue.

Phospholipide synthesis in diabetic dogs. D. B. ZILVERSMIT. *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

Half a millicurie of radioactive phosphorus was injected intravenously into depancreatized and also normal dogs. Six hours later the animals were sacrificed and samples of liver, plasma, small intestine, kidney, muscle and brain were analyzed for radioactive phospholipides. In addition, the specific activity of plasma inorganic phosphate was measured. 'Relative specific activities' of tissue phospholipides, i.e. the ratio of the phospholipide specific activity to the specific activity of plasma inorganic phosphate, were used to compare the turnover rates of phospholipides in the tissues of diabetic and normal animals. For the measurement of plasma phospholipide turnover rate, the ratio of the specific activity of

plasma phospholipide to the specific activity of liver phospholipide in the same animal was used. The relative specific activities of liver, kidney, small intestine and plasma phospholipides were found to be about twice as large in the diabetic dogs as in the control animals. In muscle or brain, however, no evidence was found of an increased synthesis of phospholipides in the diabetic dogs. These findings contribute to the evidence that some phospholipides play a role in fat oxidation. (Supported by a grant from the American Cancer Society.)

Action of choline on in vivo and in vitro synthesis of phospholipides. D. B. ZILVERSMIT AND N. R. DILUZIO.* *Div. of Physiology, Univ. of Tennessee School of Biological Sciences, Memphis.*

Choline is known to stimulate phospholipide synthesis in livers of intact rats and dogs. In accordance with Artom's findings on rat liver slices (*Federation Proc.* 9:147, 1950), we have found that *in vitro* added choline does not alter phospholipide synthesis in dog liver slices. The discrepancy between the action of choline *in vivo* and *in vitro* led to the study of intravenously administered choline on liver phospholipides. In order to introduce choline into the body at the same rate at which it is absorbed from the gastrointestinal tract, the rate of absorption of choline chloride was measured. In two dogs, 1.9 gm. of choline chloride disappeared from the gastrointestinal tract in one hour after feeding 2.9 gm. of choline chloride by stomach tube. However, the administration of this amount of choline chloride either by femoral or portal vein at a rate of 1 gm. per hour or less was uniformly fatal. Apparently, the major amount of fed choline does not reach the blood stream unchanged. Additional experiments have shown that large amounts of choline disappear from a Ringer-Locke solution during incubation with a piece of small intestine *in vitro*. The increase of phospholipides in this tissue observed during such incubation could not account for the lost choline. The action of choline on intestinal phospholipides is now under investigation. (Supported by a grant from the U. S. Public Health Service.)

Release of vasoconstrictor substance(s) from platelets. MARJORIE B. ZUCKER. *New York Univ. Dental College, New York City.*

The mechanism of the release of platelet vasoconstrictor substance(s) during clotting is not known. A study of the effect of various components of the clotting mechanism on the release of these vasoconstrictors is under way. In control studies, equal amounts of thrice washed platelets obtained in silicone glassware from Dowex-50 human blood were added to saline, to 0.0025 M $CaCl_2$ in saline, to Dowex plasma or to recalcified Dowex plasma. The final mixtures, containing approximately 75,000 platelets/mm.^{3}, were incubated at 37° C. for 45 minutes in glass tubes. They were centrifuged and frozen until tested for vasoconstrictor activity on the perfused rabbit ear. Activity in both of the saline tubes was very slight, indicating that calcium alone cannot release the constrictor material. Activity in the non-recalcified plasma-plus-platelet samples was somewhat greater, as was that of recalcified and non-recalcified plasma alone, due to liberation of vasoconstrictor material during preparation of the plasma. The samples containing calcium and plasma-plus-platelets showed significantly more activity than non-recalcified plasma-plus-platelets. The normal content and release of vasoconstrictor substances from washed platelets was further indicated by the fact that the activity of recalcified plasma-plus-washed platelets was at least as great as that of recalcified slowly centrifuged plasma. Similar results were obtained when oxalate plasma and platelets from citrated blood were used. Recalcification liberates as much vasoconstrictor material from slowly centrifuged plasma as does repeated freezing and thawing. Platelets incubated in saline plus thrombin (bovine, Parke, Davis & Company 10U/ml.) with or without calcium do not release vasoconstrictor substance(s). (Aided by Life Insurance Med. Research Fund.)

Factors concerned in the compensatory vascular readjustment to hemorrhagic shock. B. W. ZWEIFACH, S. BAEZ AND EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Med. College and New York Hosp., New York City.*

The regulation of the circulation through the terminal vascular bed is dependent upon the interplay of a number of discrete functional changes. It is the purpose of the present studies to establish, by direct visualization of the peripheral circulation in the mesentery, the relative significance of the various factors concerned. Hemorrhage experiments were carried out on dogs and rats following surgical removal of the kidneys, adrenals and/or the sympathetic nervous system, singly or in combination. The specific indices followed were the development of hyperreactivity of terminal arterioles and precapillaries, augmentation of peripheral vasomotion, and relative capacity of the terminal vascular bed to undergo selective vasoconstriction so as to maintain an effective peripheral blood flow following extensive blood loss. Removal of the kidneys resulted in the exclusion of VEM from the blood stream following hemorrhage. In contrast to the hyperreactive response of normal animals (25–100x), only a

moderate increase in reactivity (10–20x) was observed in adrenal animals following comparable blood loss. Sympathectomy interferes with normal vasomotion and considerably reduces the augmented vasomotion following hemorrhage. The combined removal of both the kidneys and the sympathetic nervous system definitely predisposed dogs to the deleterious effects of hemorrhagic shock. Removal of the adrenals impairs the ability of the terminal vascular bed to readjust following extensive blood loss. The interrelation of these 3 sets of factors and their relative significance for the shock syndrome will be discussed.

POSTHUMOUS PAPER

A theory of reflex control of temperature at rest and during exercise. H. C. BAZETT.[1] *Dept. of Physiology, Med. School, Univ. of Pennsylvania, Philadelphia.*

Reflex responses exist to warmth, as well as to cold since distant intra-arterial temperatures show reactions to both within 12 seconds. Responses to heat of external and internal origin differ. The former utilizes vasomotor adjustments, until surface temperature (T_S) exceeds 34°, when sweating supervenes; the latter utilizes sweating which is proportional to the existing Ts and maintains, rather than alters, it. Yet rectal temperature (T_R) is regulated at a raised level, gradually attained, and proportional to the work. The problem is to account for such regulation of T_R by dermal sensory mechanisms prone to adaptation. The theory assumes two groups of receptors for warmth (both Ruffini), one dermal (W_1) and one subcutaneous (W_2). Both have been identified physiologically and histologically in the prepuce. Similar groups, demonstrated histologically in standard skin by Wollard *et al.* are assumed to be concerned in the control, with vasomotor adjustments induced by external warmth through W_1, and sweating by deep warmth through W_2. The apparent relationship of sweating to T_S is due to its dependence on thermal gradients for sensation and on T_R for level. T_S also varies with both, for $T_S = T_R - (T_R - T_S)$. Subnormal gradients in the dermis probably account for W_1 sensations; they are observed not to develop with vasodilatation, if surface heat loss is adequate. Deep W_2 receptors may react to venous blood warmed to temperatures exceeding those in local arteries, for warming of venous blood is an adequate stimulus in the prepuce. The critical level of T_S for sweating at rest might depend on warmed muscular blood inducing such a local reversed gradient, if it mixed with skin blood which was not too cold, and the changes in exercise on the increased heat of blood from active muscles. Increased tissue gradients are needed for increased heat transport in the absence of vasomotor effects by W_2, which results in raised T_R, until potentiated reflexes from W_1 achieve a balance. On such assumptions sweating rates may be predicted for a single subject over a wide range of environments and metabolic rates. The data support, but do not prove, the hypotheses.

[1] Deceased.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

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Biochemical changes induced by vitamin B_{12}. LYNN D. ABBOTT, JR. *Dept. of Biochemistry, Med. College of Virginia, Richmond.*

Nitrogen and phosphorus balances before and after injection of vitamin B_{12} in rabbits on both unrestricted and constant dietary intake have been studied. In rabbits on unlimited intake of Rabbit Chow Checkers, injection of B_{12} (5 μg/day for 5 or 6 days) was accompanied by marked positive nitrogen balance. The animals ate more and retained more nitrogen than in preceding control periods. Phosphorus retention also occurred. The ratio of P:N retained under the influence of B_{12} approximated Benedict's P:N ratio for protoplasm (1:14). When B_{12} administration was stopped balances gradually decreased to former levels. In animals on a constant intake containing 2.5 gm. N per day, slight positive nitrogen balance occurred under the influence of B_{12} even though additional food was not available during this period. On a constant diet of oats and cabbage which furnished a low N intake, urinary excretion of nitrogen, urea, amino acids and phosphorus diminished during B_{12} administration. These results indicate that in the normal rabbit under these conditions increased utilization of nitrogen and phosphorus occurred.

Phosphoribomutase and enzymatic preparation of ribose-1-phosphate. ADOLPH ABRAMS AND HANS KLENOW (introduced by H. M. KALCKAR). *Inst. for Cytophysiology, Univ. of Copenhagen, Copenhagen, Denmark.*

The soluble protein fraction of rat liver contains an enzyme, phosphoribomutase, which converts acid labile ribose-1-phosphate to an acid stable phosphate ester, presumably ribose-5-phosphate. The existence of this enzyme had been suggested by Schlenk (*Adv. in Enzymology* 9: 473) and experimental evidence for its presence in muscle extracts was obtained by Kalckar (unpublished experiments). When inosine and orhtophosphate are incubated with rat liver extracts, the nucleoside phosphorylase causes the formation of ribose-1-phosphate (KALCKAR. *J. Biol. Chem.* 167: 477, 1947) which is then converted to an acid stable phosphate ester through the action of phosphoribomutase which is also present in the crude extract. The phosphoribomutase activity can be measured directly by determining the rate of disappearance of acid labile phosphate when ribose-1-phosphate is incubated with the enzyme preparation. Attempts to prepare ribose-1-phosphate by the action of crude nucleoside phosphorylase on inosine and orthophosphate usually resulted in a very low or no yield because of the presence of phosphoribomutase whose activity was comparable to that of the nucleoside phosphorylase. We have found that phosphoribomutase can be inactivated by surface denaturation due to foaming. It was possible to obtain a fairly good yield (38%) of ribose-1-phosphate when the diluted enzyme preparation was preliminarily foamed by bubbling air or nitrogen, which left the phosphorylase relatively intact while the phosphoribomutase usually was nearly completely destroyed.

Proteolytic activity of pituitary extracts. ELIJAH ADAMS (introduced by EMIL L. SMITH). *Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.*

Aqueous extracts of hog or beef pituitaries (lyophilized and acetone powders of entire, anterior and posterior glands) contain a variety of exopeptidases. Proteolytic coefficients for hydrolysis of glycyl-L-proline (prolidase) and L-leucinamide (aminopeptidase) are comparable with those for extracts of hog duodenal mucosa. Hydrolysis rates for related substrates and metal-activation characteristics indicate the similarity of these enzymes in pituitary and other tissues. Additional enzymes include those which split triglycine and glycylglycine; hydrolysis of the latter is activated by Co^{++} as in extracts of other tissues. Aqueous extracts of lyophilized anterior swine pituitary digest horse hemoglobin as measured by the Anson-Kunitz method. Two proteinases can be distinguished which have optimal activity at pH 3.8 and pH 8.3, and by phosphate inhibition of the latter enzyme. Each enzyme has been partially purified and separated relatively free of the other, the pH 3.8 enzyme in the supernatant from pH 5.5 precipitates of crude extract,

and the pH 8.3 enzyme by differential heat inactivation. Both partially purified preparations also hydrolyze edestin, human serum albumin and γ-globulin. These proteinase actions are not activated by the usual metal ions or by cysteine. Thus far, neither carboxypeptidase nor endopeptidase activities have been observed with known synthetic substrates. Purified hog ACTH, prepared by the method of Sayers, White and Long, hydrolyzes hemoglobin at the pH optima characteristic for each proteinase. Although the contaminating proteinases in ACTH are present in minute amount, they may be responsible for ACTH instability under certain conditions.

Isolation of conjugated and free bile acids by extraction procedures. E. H. AHRENS, JR.* AND LYMAN C. CRAIG. *Rockefeller Inst. for Med. Research, New York City.*

Isolation of conjugated and free bile acids from other components of bile and their separation from each other has been accomplished by countercurrent distribution. The following conclusions have been reached: *1*) Extraction of lyophilized unhydrolyzed bile with absolute alcohol was the simplest procedure tested. *2*) Quantitative separation of bile acids from other components could not be achieved by classical extraction with ether or hydrocarbon solvents because of association tendencies. *3*) This difficulty was overcome by countercurrent distribution in preparative runs in the system, glacial acetic acid/*n*-heptane, with 20 transfers. *4*) Bile acids conjugated to taurine were separated from glycine conjugates, free acids, and phosphatides after 30 transfers in the system, *n*-butanol (30 o/o), *n*-heptane (70 o/o)/acetic acid (10 o/o). *5*) Taurine conjugates were separated from each other in 2-butanol/acetic acid (3 o/o). *6*) Glycine conjugates and free acids were individually isolated in isopropyl ether (85 o/o), *n*-heptane (15 o/o)/acetic acid (60 o/o). In this system phosphatides (K=10) were separated from the bile acids (K = <2). *7*) The above systems take advantage of marked differences in polarity of the various bile acids, ranging from the most hydrophilic compound, taurocholic acid, to the least, lithocholic acid. Tauro-acids are much more polar than glyco- or free acids. The presence of 1, 2 or 3 −OH groups on the cholanic acid nucleus also markedly influences partition ratios. The keto-derivatives are more polar than the corresponding hydroxylated compounds. *8*) The green color of bile may be due to concentrated solutions of the conjugated bile acids rather than to bilirubin products.

Biological value of enzymatic digest of bovine plasma. ANTHONY A. ALBANESE, REGINALD A. HIGGONS,* GEORGE E. MACDONALD,* WILLIAM C. FELCH,* BETTY VESTAL* AND LOUKIA STEPHANSON.* *Nutritional Research Lab. and Dept. of Medicine, St. Luke's Hosp., New York City.*

The nutritional value of an enzymatic digest of bovine plasma (Travamin) was tested in young children and convalescent adults. It has been found that the nitrogen retention and weight gains of male infants on a synthetic milk in which the bovine plasma digest constituted the principal source of dietary nitrogen were equal, if not superior to those obtained on an evaporated milk formula fed at the same fluid, caloric distribution and nitrogen levels. The nutritional value of the hydrolysate was not improved by reinforcement with isoleucine and methionine. Parenteral assays of the digest in 6 convalescent adult males showed an average utilization of 86.3% of the infused nitrogen. This compares favorably with the utilization of various protein hydrolysate and amino acid preparations reported by others. Most of the nitrogen which escapes utilization was found in the urine in the form of free and bound amino N. The possibility that the nutritional value of N derived from sources which include peptides may be greater than an equivalent amount of N in the form of a mixture of free amino acids is discussed.

Protein sparing action of various carbohydrates. ANTHONY A. ALBANESE, REGINALD A. HIGGONS,* LOUKIA STEPHANSON* AND BETTY VESTAL.* *Nutritional Research Lab., St. Luke's Hosp., New York City.*

Recent observations on the successful parenteral use of invert sugar has created the need for a critical evaluation of the protein sparing action of the different carbohydrates occurring in the human dietary. In an attempt to fill a part of this need, studies were made on 8 normal children (7–13 yr.) fed chemically defined diets at the rate of 60–80 calories/kg. (protein, 10%; fat, 35%; and carbohydrate 55%) containing varying amounts of lactose, glucose, sucrose, fructose, dextrose, and dextrins. These measurements have shown that N-retention and utilization was greatest with diets which provided approximately 350 mg. of protein N/kg. and contained 70% of the total carbohydrate fraction as fructose. An isocaloric reduction of the protein N intake to 200 mg/kg. diminished or voided the exceptional N sparing effect of the high fructose diets. Details of the N-sparing action of carbohydrates as revealed by chemical and chromatographic amino acid analyses of the blood and urine collected during these studies will also be discussed.

Change in adenine nucleotides during growth in plant tissues. HARRY G. ALBAUM, ALTA HIRSHFELD,* THEODORE CAYLE* AND FRANCIS BON-

NER.* *Biology Research Lab., Brooklyn College, Brooklyn, N. Y.*

Studies have been carried out on changes in adenine nucleotide during growth of mung beans. The seeds were soaked for 5 hours in water and germinated for 1, 2, 3, and 4 days at 28°C. in the dark. Immediately after soaking and each day thereafter, equal weights of seedlings (approximately 3 gm. wet weight) were extracted with trichloracetic acid. The acid soluble phosphorus was precipitated with barium and alcohol and ultimately assayed for adenylic acid, adenosine diphosphate and adenosine triphosphate spectrophotometrically using enzymes. Adenine nucleotide was present largely as ATP. The value increased sharply from the time of soaking to the first day. Thereafter it remained fairly uniform; this in spite of the fact that the seedlings were growing very rapidly. The adenine nucleotide content of these seedlings was then assayed by using the chromotographic procedure of Carter and Cohn. In contrast to the results obtained above, a fraction which assayed as ATP chemically showed almost a linear increase between zero and 3 days. Throughout the period studied, the ATP which could be detected enzymatically was only a part of the total ATP which was present. These results are in conformity with the data obtained on the isolation of ATP from these tissues. Finally, studies have been carried out on the incorporation of radiophosphorus into the adenine nucleotides of the growing plants. More radiophosphorus is incorporated when the plant is growing rapidly as compared to when it is growing slowly.

Incorporation of deuterium into plasma and liver cholesterol of the rat under different dietary conditions. ROSLYN B. ALFIN-SLATER,* MICHAEL C. SCHOTZ,* FRANK K. SHIMODA,* AND HARRY J. DEUEL, JR. *Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Southern California, Los Angeles.*

The effect of high and low fat diets on normal rats and on rats previously fed a high cholesterol diet was studied by measuring the uptake of deuterium by cholesterol in the liver and plasma of rats placed on these different diets for various lengths of time. A constant deuterium oxide concentration in the body fluids was maintained by an initial injection of 25% deuterium oxide and subsequently allowing the animals to drink a diluted deuterium oxide solution. Total cholesterol was determined by a modified Schoenheimer-Sperry procedure, then isolated as digitonide, combusted, reduced and the deuterium-hydrogen ratio read in an Isotope-Ratio Mass Spectrometer. The results indicate that the amount of cholesterol synthesized in the liver of the rats under the different dietary conditions imposed was the same regardless of the differences in the pre-experimental and experimental diets. The amount of labeled cholesterol in the plasma did not differ with the experimental high and low fat diets but varied with the pre-experimental dietary conditions. A high fat diet seems to be more efficacious in removing from the liver, cholesterol accumulated there by a high cholesterol diet.

Possible existence of ribose non-esterified at positions 2 and 3 in ribonucleic acids and certain fractions derived by action of ribonuclease. FRANK WORTHINGTON ALLEN. *Dept. of Biochemistry, Univ. of California Med. School, Berkeley.*

Oxidation by periodate has been used to detect the possible existence of ribose non-esterified at positions 2 and 3 or in the following substances: *a*) commercial ribonucleic acids, *b*) ribonucleic acids purified by chemical procedures, *c*) purified, dialyzed ribonucleic acid recovered by lyophilization, *d*) the dialyzates collected serially from the action of ribonuclease upon *c*, and *e*) the non-dialyzable residue from the action of ribonuclease upon *c*. Both *a* and *b* show highly variable oxidation by periodate. The substances that are responsible for the major part of the oxidation are dialyzable. Results show that *c* contains no more than one ribose molecule that can be said to be non-esterified at position 2 or 3 per 25–35 molecules of esterified phosphate. Results for *d* indicate that the action of ribonuclease is concerned with at least two types of linkages. The first collected dialyzates wherein the ratio of purine-containing constituents to pyrimidine-containing constituents is 2:3 show the possibility that 1 mole of ribose may be non-esterified for every 15 moles of phosphate. As the ratio of purine-containing constituents in the dialyzate increases over that of the pyrimidine-containing constituents the ratio of non-esterified ribose per mole of phosphate becomes approximately 1:2. Data for *e* reveal little utilization of periodate such that no more than 1 mole of non-esterified ribose per 30–40 moles of phosphate can be present.

Biochemical studies on pancreatic function. Serum lipase activity. C. ALPER AND P. P. POLAKOFF, JR. (introduced by M. J. BOYD). *Div. of Biological Chemistry, Hahnemann Med. College, Philadelphia, Pa.*

The determination of serum lipase activity may be simplified by the use of a fat emulsion as the substrate. Clinical determinations of serum lipase have been unsatisfactory because of the wide range of normal activity. Lipolytic activity of individual and pooled human sera and pooled horse sera were determined by two methods. The results obtained using a tributyrin emulsion

yielded values which were higher and more constant than those obtained using a tributyrin dispersion. The units were expressed as milliliters of 0.1 N fatty acid released in one hour per 100 ml. of serum. The results obtained with pooled human sera and horse sera were, respectively, 73.5 (6) and 130 (12) U/100 ml. of serum. The results obtained using the tributyrin emulsion with individual human sera and pooled horse sera were, respectively, 124 (26) and 168 (12) U. The emulsion and dispersion were of similar composition except that the former contained Tween 20 in addition to barbiturate buffer, (pH 8.4) calcium acetate and tributyrin. The emulsion was stable for several weeks after preparation. Studies on the effect of pH control on the reaction revealed that, with barbiturate buffer horse serum lipase activity was constant within a range of pH 8 ± 1; with phosphate buffer the activity increased as the buffer became more acid, 67 (5), 91 (5), and 109 (5) U pH 8.35, 7.4, and 6.9, respectively.

Variations in alkali resistant hemoglobin in normal and abnormal bloods. MARIE A. ANDERSCH AND ANNA MAE ROSENDALE.* *Dept. of Medicine, Subdept. of Clin. Pathology, Univ. of Maryland School of Medicine, Baltimore.*

The rate of the alkali denaturation of hemoglobin obtained from normal individuals in different age groups was compared with the denaturation rate of hemoglobin found in the cells of patients with various blood dyscrasias. The spectrophotometric method of Haurowitz was adapted for use with the Beckman B spectrophotometer, and the percentage transmission was measured at intervals following the addition of N/4 sodium hydroxide to a hemoglobin solution. The sharp breaks in the denaturation rate seems to indicate the presence of more than two hemoglobins in normal blood. Some patients with certain hemolytic anemias showed an increase in an alkali resistant hemoglobin; the most marked increase being found in a patient with Mediterranean Anemia.

Absorption and metabolism of tyrosine disulfonate. JAMES C. ANDREWS AND JOHN P. FEASTER.* *Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill.*

Tyrosine 3-5 disodium disulfonate has been prepared and administered to rabbits. This compound was investigated because it represents an amino acid sulfonate the metabolism of which has not previously been studied and because it might give some information regarding the degradation products of tyrosine. The compound was prepared by direct sulfonation of tyrosine in concentrated sulfuric acid at 65°C. The barium salt was prepared and purified and from this the free acid and the sodium salt were made. The physical properties of these compounds (solubility, optical activity and titration curves) were determined. The sodium salt was administered to adult male rabbits orally, subcutaneously and intravenously, the dosages ranging from 0.4–1.5 gm. in terms of sulfur content. There was no indication of toxic effects. Normal and post-administration urine samples were analyzed for total sulfur, total sulfate-sulfur, creatinine, total nitrogen, and urea nitrogen, and total sulfur values were determined in fecal samples. Following intravenous and subcutaneous injections, the urine showed an increase in total sulfur content amounting to about 70% of the sulfur administered, with no increase in the sulfate-sulfur fraction. The compound was excreted unchanged in the urine. Oral administrations were followed by a corresponding increase in fecal total sulfur but no alteration of urinary sulfur values. In sharp contradistinction to other sulfonates (such as cysteic acid) the compound is absorbed only with great difficulty.

Evidence for stepwise biosynthesis of ovalbumin. CHRISTIAN B. ANFINSEN AND DANIEL STEINBERG.* *Dept. of Biochemistry, Harvard Med. School and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Samples of crystalline, ovalbumin containing C^{14}-carboxyl labelled tyrosine, alanine, glutamic acid or aspartic acid were prepared by incubating minces of hen's oviduct with solutions containing the corresponding labelled amino acid or with $C^{14}O_2$. Physical and immunochemical tests on these preparations indicated the absence of protein impurities. Tests for adsorbed radioactive non-protein materials were also negative. By employing the specific proteolytic reaction described by Linderstrøm-Lang for the conversion of ovalbumin to plakalbumin, plus non-protein fragments, a single radioactive aspartic acid residue could be separated from those remaining in the plakalbumin molecule. The specific radioactivity of the CO_2 released by ninhydrin from this aspartic acid was from 2–4 times as great as that derived from the average aspartic acid molecule in the plakalbumin. These results make unlikely an all-or-none template synthesis of ovalbumin from a free amino acid pool. They support a process involving stepwise synthesis from preformed intermediates. Amino acid exchange along the protein chains cannot be ruled out, however. Labelled ovalbumin preparations were also subjected to peptic digestion. The various peptide fragments produced were then partially separated by ion exchange chromatography and counter-current distribution. After hyrolysis of these fractions, samples of carboxyl CO_2 were

obtained by enzymatic decarboxylation or by ninhydrin treatment of the isolated amino acid. The observed variation in the specific radioactivities of the carboxyl carbons obtained in this manner add further support to the hypothesis of stepwise protein synthesis.

Free choline in plasma. HAROLD D. APPLETON,* BETTY B. LEVY,* J. MURRAY STEELE* AND BERNARD B. BRODIE. *New York Univ. Research Service, Goldwater Memorial Hosp., New York City, and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Using a specific micromethod for the estimation of free choline previously reported (*Federation Proc.* 9: 146, 1950), plasma values for normal adults were found to range from 2–20 μg. per ml. In spite of this wide range, the plasma levels for the same individual remained essentially constant over a period of 9 months. The findings suggest that free choline in plasma is maintained constant by some regulatory mechanism, the significance of which is not yet understood. Patients representative of various disease states had values within the normal range except for a few with kidney disease whose values were in the upper normal range or higher. The plasma choline level in dogs was raised to approximately twice the normal value for a period of 1–3 hours by means of a constant intravenous infusion of choline at a rate of 3.4 mg. per minute. Within one hour after stopping the infusion, the plasma choline had returned to the normal level. About 20% of the injected choline was accounted for in the urine. The fate of the remainder is now under investigation. At normal plasma levels, little if any choline was excreted in urine. At the elevated choline levels, however, a significant amount of the compound was excreted. To what extent the kidney participates in the regulation of the level of choline in plasma is now under investigation.

Distribution of rhodanese in blood. REGINALD M. ARCHIBALD, RUTH MEYER* AND JAMES GREENOUGH.* *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

Some workers report that rhodanese is present in erythrocytes; others that it is absent from blood. Human blood leukocytes and erythrocytes were partially separated by centrifugation in 30% sucrose. Rhodanese measurement by the Rosenthal *et al.* method (except for 1 hr. incubation at 30°C.) revealed no detectable amount in human plasma and an appreciable amount in leukocytes. In each human blood the ratio of activity found in the leukocyte fraction to that in the erythrocyte fraction was proportional to the number of leukocytes in the two fractions. Normal human erythrocytes have (if any) less than 5% of the total rhodanese of whole blood and at least 95% (and perhaps all) of the rhodanese in normal human blood is in the leukocytes. Rhodanese concentration in liver homogenates was not increased by restriction of mice for two weeks to 2% sodium thiosulfate as drinking fluid and dry food soaked in 2% KCN solution. The mice ate well. More than half of the rhodanese in rat liver homogenates is found after differential centrifugation in 30% sucrose, in the mitochondrial fraction. Incubation of fresh rat liver homogenate with sulfite and thiocyanate at pH's 6.5, 7.4, or 8.1 did not cause appreciable disappearance of thiocyanate despite the reported ability of sulfite to inhibit markedly conversion of cyanide to thiocyanate by rhodanese. The concentration of rhodanese (and arginase) in rat liver was not changed appreciably by marked hypothyroidism induced by prolonged feeding of propyl thiouracil or by thyroid destruction from administration of I^{131}.

Availability of L- and D-phenylalanines for growth. MARVIN D. ARMSTRONG AND JOY D. LEWIS (introduced by F. BINKLEY). *Univ. of Utah College of Medicine, Salt Lake City.*

A comparison has been made of the ability of L- and D-phenylalanines to promote the growth of young white rats receiving synthetic diets deficient in phenylalanine. In addition, each of the two isomers was fed to young rats at high levels in the diet to see if a toxic effect of either could be demonstrated. Poorer growth was obtained with diets which contained D-phenylalanine than was found with those containing the same amounts of the L-isomer. This effect was especially marked at levels of the compounds corresponding to the minimum at which good growth was obtained with L-phenylalanine. *Ad libitum* feeding was used for these experiments and an apparently greater palatability of diets containing the L-isomer resulted in a much higher consumption of it. Decreased food consumption thus appears to be the main factor responsible for the slower growth found with D-phenylalanine. There was no sign of a significant toxicity of either isomer when as much as 4.0% was included in the diet.

Incorporation of labeled choline in phospholipides of isolated liver tissue. CAMILLO ARTOM, MARIETTA CROWDER* AND MARJORIE A. SWANSON. *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*

C^{14} labeled choline chloride was added to slices or homogenates of liver from rats previously maintained on various diets. After 3½ hours of incubation in Krebs-Ringer-phosphate (pH 7.4), the lipides were extracted and their contents of C^{14}, choline and phosphorus were determined.

In oxygen the percentage of added choline incorporated into the phospholipides is much smaller with slices of liver from rats on stock diet than with slices of liver from rats maintained on low protein diets, with or without added guanidoacetic acid. Substitution of nitrogen for oxygen decreased the incorporation of choline in the lipides of the liver from rats on the low protein diets, but not from rats on stock diet. In both groups of animals homogenization of the liver reduced markedly, but did not suppress completely, the uptake of labeled choline by the lipides. *In vitro* addition of ethanolamine and diethanolamine (0.003 M; rats on stock diet; slices in O_2) inhibited, whereas addition of betaine *in vitro* enhanced, the process. A comparison between the incorporation of labeled phosphate and labeled choline in the phospholipides of isolated tissues suggests that formation of the linkage between choline and the phosphatidic acid moiety may occur to a certain extent independently of the formation of the other ester bonds in lecithins. (Work performed under Contract AT-(40-1)-288, Title II, with the U. S. Atomic Energy Commission.)

Biological vitamin C activity of the *Malpighia punicifolia* L. pseudo cherry. CONRADO F. ASENJO. *Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Puerto Rico, San Juan.*

Earlier studies on this pseudo cherry (ASENJO AND GUZMÁN, *Science* 103: 219, 1946) indicated that its ascorbic acid content ranged between 1000–3000 mg/100 ml. of juice. Isolation of the crystalline acid was accomplished. The present study was undertaken to determine if the biological activity of the juice was comparable with its chemical assay value. Guinea pigs kept for a preliminary period of two weeks on the Sherman La Mer Campbell diet supplemented daily with 1 mg. of synthetic ascorbic acid were divided into 3 groups of 4 each. One group was supplemented with 0.1 ml/day of the juice from ripe fruits, a second group with 1 mg/day of synthetic ascorbic acid and a third group as negative control. The trial lasted until the last of the controls died (4 weeks). The animals on the cherry juice gained 35 gm. and those on the ascorbic acid 29 gm. The negative controls lost 68 gm. A second experiment performed with 2 animals indicated that 0.1 ml. of juice as supplement cured the deficiency even when the animals had lost as much as 65 gm. of body weight during the depletion period. On the basis of these results, the juice of the ripe *Malpighia punicifolia* L. pseudo cherry should contain at least 1000 mg. of vitamin C/100 ml. of juice. Assays with 2,4 dinitrophenylhydrazine averaged: ascorbic acid 1848 mg., dehydroascorbic acid 482 mg., total 2330 mg/100 ml. of juice.

Hydrolysis of procaine and 2-chloroprocaine in spinal fluid. MANUEL H. AVEN AND FRANCIS F. FOLDES (introduced by L. REINER). *Dept. of Anesthesia, Mercy Hosp. and Dept. of Anesthesia, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

0.05 to 0.60 mg/cc. of procaine or 0.20 mg/cc. of 2-chloroprocaine was added to spinal fluid immediately after its withdrawal. The solutions were kept at 37° C. for 3 hours and then analyzed for procaine and *p*-aminobenzoic acid, and 2-chloroprocaine and 2-chloro-4-aminobenzoic acid, respectively (spectrophotometric method of TING, COON AND CONWAY. *J. Lab. & Clin. Med.* 34: 822, 1949). In 3 hours about 10% of the procaine and 40% of the 2-chloroprocaine was found to be hydrolyzed. The percentage of procaine hydrolyzed seemed to be independent of the initial concentration. Heat inactivation of the spinal fluid (30 minutes at 56° C.) or the addition of various enzyme inhibitors (neostigmine or dimethylcarbamate of 2-hydroxy-5-phenylbenzyltrimethyl ammonium bromide) had no effect on the rate of hydrolysis. These findings indicate that there is very little or no procaine esterase in spinal fluid. In contrast to this, under similar experimental conditions, 0.2 mg/cc. of procaine, or 2-chloroprocaine in the plasma of the same patients was completely hydrolyzed in 20 and 4 minutes, respectively. This hydrolysis could be markedly inhibited by heat inactivation, or the addition of the above enzyme inhibitors.

Phosphate uptake by pea meal extracts. BERNARD AXELROD, ROBERT S. BANDURSKI* AND PAUL SALTMAN.* *Enzyme Research Div. Bureau of Agricultural and Industrial Chemistry, U. S. Dept. of Agriculture, Albany, Calif. and Kerckhoff Labs. of Biology, California Inst. of Technology, Pasadena.*

A particulate-free extract of pea meal was incubated with radioactive phosphate and the products resulting from the esterification of the endogenous substrate isolated as the barium-insoluble and barium-soluble fractions (UMBREIT, BURRIS AND STAUFFER, *Manometric Techniques*, 1945). By the use of two-dimensional chromatography the following labeled compounds were isolated: Glucose-1-phosphate, phosphoenolpyruvate, fructose-1,6-diphosphate, adenosine-5-phosphate, probably fructose-6-phosphate, two unknown barium-insoluble compounds and one unknown barium-soluble compound. A net increase in the fructose-1,6-diphosphate was observed by the conventional analytical method. In addition, a substance which possessed mobilities identical to adenosine triphosphate had become labeled. No 3-phosphoglyceric acid could be detected. In all cases unlabeled authentic compounds

were co-chromatographed with the reaction products and identification made by superposition of the radioautographs on the developed chromatograms.

Cytochrome system of *Trypanosoma cruzi*. H. D. Baernstein and E. J. Tobie.* *Natl. Insts. of Health, Bethesda, Md.*

Spectroscopic observation of thick suspensions of the culture forms of *T. cruzi* showed, in the presence of glucose, a strong band at 556 mμ and a weaker one at 532 mμ. A questionable band at about 600 mμ was too weak to study. There was no band at 550 mμ representing cytochrome *c*. Shaking the sample with oxygen weakened the bands temporarily, showing reversibility. When cyanide was present, however, oxygen did not bleach the bands and there was no displacement, indicating the presence of an independent oxidase. Added cytochrome *c* was immediately reduced but could not be oxidized by the oxidase present. This differentiates the oxidase of *T. cruzi* from the oxidase of yeast. Another difference between these oxidases is the failure of 95% carbon-monixide-5% oxygen mixtures to inhibit the oxygen uptake in the Warburg apparatus in the dark. In the presence of urethane the bands did not bleach with oxygen, the same as in the case of cytochrome *b*. The cytochrome of *T. cruzi* also resembles cytochrome *b* of yeast in being insoluble and in not being reduced by hydroquinone, ascorbate, thioglycollate, and paraphenylendiamine. Hydrosulfite reduced both. Neither lactate nor succinate reduced cytochrome of *T. cruzi* probably owing to the absence of the requisite dehydrogenases. Sodium hydroxide and pyridine do not alter the spectrum significantly and therefore the prosthetic group is probably not protohemin.

Excretion of bromsulphthalein and sodium cholate after intravenous injection. Harold W. Baker,* Carl E. Anderson and Robert H. McCluer.* *Depts. of Biochemistry, Vanderbilt Univ., Nashville, Tenn., and Univ. of North Carolina, Chapel Hill.*

The rate of excretion by the liver of cholic acid and bromsulphthalein (BSP), separately and combined, was studied in normal and liver damaged dogs. The sodium cholate solution, adjusted to pH 7.4, was passed through a Selas porcelain filter and a dose of 50 mg/kg/body weight used. BSP was administered at a rate of 5 mg/kg/body weight. For the combined cholate and BSP experiments the two solutions were mixed before being injected. Cholic acid was determined by a modification of the method of Minibeck and BSP by the method of Gaebler. In normal dogs the rate of excretion of cholate, and of BSP separately, agreed closely with the findings of others. When the two substances were administered in combination the excretion of BSP was greatly retarded for the first 15 minutes while the rate of excretion of cholic acid was not significantly altered. In early central necrosis of the liver produced by carbon tetrachloride the rate of excretion of BSP was definitely delayed while the cholic acid rate was only slightly retarded. When the combined solutions were injected into the dogs with liver damage the BSP retention values roughly paralleled the curve obtained by BSP alone but were 35–60 % higher. The effect was not due to order of injection or mixing. It would appear that in the dog the liver preferentially excretes the cholate before excreting BSP.

Nutritional studies with hexahydrophenylalanine. Bernard J. Baltes. (introduced by Edward A. Doisy). *Dept. of Biochemistry, Saint Louis Univ. School of Medicine, Saint Louis, Mo.*

Investigation of a structural analog of phenylalanine (PA) was undertaken to ascertain if either antagonism or dehydrogenation to PA occurs. Hexahydrophenylalanine (HPA) was synthesized by a modification of the procedure of Brauchli and Waser (*Helvet Chim. Acta* 7: 740, 1924). Weanling albino rats were handled with the customary techniques. The source of dietary nitrogen was crystalline amino acids. The control diet (I) containing 1% PA fed over the 30-day experimental period resulted in 62 gm. gain in body weight and 190 gm. of I consumed (averages for the group). Incorporation in I of 1% HPA (II) led to decreased growth rates and food consumption (19 and 122 gm., respectively). To minimize the influence of anorexia, paired feeding technique was employed (Restricted I). The rats on Restricted I were permitted identical amounts of food and gained 28 gm., while the animals on II gained only 19 gm., indicating not only an appetite-depressant but also a toxic action of HPA, perhaps antimetabolic in nature. Doubling PA in II did not lead to significant increases in weight or food consumption (22 and 120 gm.) while doubling HPA in II resulted in no weight gain and lowered food intake. Dietary and injected HPA produced in every animal a characteristic syndrome; growth retardation, wetting of the periurethral skin and hair, followed by alopecia localized to the perineal region, and extensive porphyrin deposits on the facies and extremities.

Distribution of radioactive thallium in chicken embryos and rats. Ralph K. Barclay, Wendell C. Peacock and David A. Karnofsky (introduced by Aaron Bendich). *Divs. of Exptl. Chemotherapy and Physics, Sloan-Kettering Inst., New York City.*

Radioactive thallium (Tl^{204}, a β-ray emitter, ½ life, 3½ years) was injected as $Tl_2^{204}SO_4$ into 4- and 8-day-old chick embryos and into adult male rats. In the fertile eggs the compound was injected both into the yolk sac and into the chorioallantoic membrane. The embryos were allowed to develop to the 18th–20th day, and then were sacrificed, and the egg contents and the embryo analyzed for Tl^{204} content. Data will be given on the distribution of the radioactive thallium when given with varying amounts of a non-radioactive carrier. Rats were given $Tl_2^{204}SO_4$ intravenously and sacrificed at various time intervals thereafter. Urine and feces samples were collected at intervals in order to follow the pattern of excretion. Four days after administration, approximately 22% of the administered dose of Tl^{204} had been excreted. After sacrificing the rats, the metal was found concentrated for the most part in bones, muscle, and kidney. Data will be given on the distribution of radioactive thallium in the rats.

Studies on mechanism of nitrogen storage. VI. Plasma proteins in induced states of nitrogen storage. PAUL D. BARTLETT. *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

Induction of nitrogen storage in laboratory animals by the administration of anterior pituitary growth hormone offers a convenient experimental procedure for studying the processes involved in protein metabolism. Utilizing this approach, we have undertaken to determine the effect of small daily doses of growth hormone on the plasma proteins of the adult female dog. Results of a preliminary study show no significant change in the concentration of total plasma proteins during induced nitrogen storage. Total plasma proteins averaged 5.46 gm.% during the control period and 5.56 gm.% during a 5-day period of induced nitrogen storage. Plasma proteins separated during the control period into multiple-component fractions by a modification of Method 10 described by the Harvard group resulted in an average value of 2.91 gm.% protein in the albumin-rich fraction, 2.11 gm.% protein in the globulin-rich fraction, and 0.26 gm.% protein in the fraction described as containing other small proteins, peptides, and non-protein nitrogen. Similar fractionations conducted on the 4th and 5th days of induced nitrogen storage gave average values of 3.13, 2.45, and 0.18 gm.%, respectively. The small protein fraction thus decreased approximately 30% while the albumin-rich fraction increased 7.5% and the globulin-rich fraction 16%. Determination of the uptake of ingested N^{15} in the multiple-component fractions separated at the peak of nitrogen storage is now in progress and will be reported.

Influence of adrenalectomy and adrenal cortical hormones in vivo on metabolism of glycine in the liver. A. D. BARTON (introduced by VAN R. POTTER). *McArdle Memorial Lab., Med. School, Univ. of Wisconsin, Madison.*

Following the intravenous or intraperitoneal injection of glycine-2-C^{14} (2–3 μg/gm. of body weight) into normal Rockland strain white mice, a large part of the injected glycine is rapidly removed from the blood by the liver, as evidenced by an abrupt rise in the specific activity of the uncombined glycine in this organ. This 'extra' glycine is disposed of in part by incorporation into liver protein and in part by catabolism of the glycine, as evidenced by the rapid appearance of $C^{14}O_2$ in the breath. Administration of a small quantity of *p*-aminobenzoic acid intraperitoneally together with the labeled glycine greatly reduces the initial burst of $C^{14}O_2$ which appears in the breath; the decreased catabolism of glycine associated with the rapid use of glycine for *p*-aminohippuric acid synthesis in the liver supports the view that the liver is the chief site of the rapid catabolism of glycine which produces the initial burst of $C^{14}O_2$ in the breath. In the adrenalectomized mouse, the 'extra' glycine is not catabolized in the liver and preliminary results with labeled tryptophan suggest that this failure to catabolize amino acids is not confined to glycine. When a small dose of adrenal cortical extract is administered to a normal mouse or to an adrenalectomized mouse before the glycine injection, the incorporation into liver protein is inhibited and catabolism of the glycine is increased. It appears that the adrenal cortical hormones exert an inhibitory effect on protein synthesis in the liver.

Relative affinity of thyroid for Br, I and Re. EMIL J. BAUMANN, NORMA Z. SEARLE, A. AARON YALOW, EDWARD SIEGEL AND S. M. SEIDLIN. *Montefiore Hosp., New York City.*

Solutions containing known activities of 2 radioisotopes of elements of the seventh periodic group (Br^{82}, I^{131}, Re^{186}) with varying amounts of carrier were injected intraperitoneally into rats maintained on a diet containing 1% of thiouracil to inhibit synthetic processes. The rats were sacrificed 1 hour later. The fraction of each element taken up by the thyroid was determined in most instances both by (*1*) chemical separation of the two isotopes and (*2*) by analysis of the composite decay curve of the total activity into its two components. For Re-Br mixtures containing nearly equal amounts of carrier for each isotope, the percentage of the injected dose of Re* found in the thyroid, 2–10%, was 60–200 times as great as that of Br. When 6–12 times the weight of carrier Br as Re was used, the fraction of Re taken up by the thyroid was 5–12%, about 25 times as

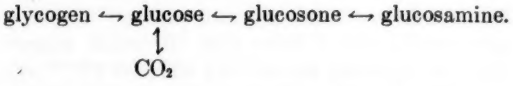
great as the fraction of Br*. With I* -Br* mixtures having varying amounts of each carrier, thyroids containing 3–11% of the injected dose of I and 0.06–0.5% of the injected Br. Using Re-I solutions, mostly with equal weights of carrier, the thyroids took up 3–15% of Re* and 4.5–12% of I*. In no case did the uptake exceed 2½ times that of Re. The affinity of the thyroid for these elements and its evident relation to atomic mass will be discussed. (Aided by a U. S. Public Health Service grant.)

Observations on plasma glutamic acid. J. R. Beaton and E. W. McHenry. *Dept. of Public Health Nutrition, Univ. of Toronto, Toronto, Ontario, Canada.*

Plasma glutamic acid levels have been determined, by the chemical procedure of Prescott and Waelsch, for normal male and female subjects, for normal pregnant women, for pregnant women with severe nausea and vomiting, and for subjects with malignant and benign growths. The mean plasma level for the normal male group was significantly higher than that for the normal female group. The mean level for the normal pregnant group was equal to that of the normal non-pregnant female group. The mean plasma level of glutamic acid was elevated well above normal in subjects with malignant growths, and in pregnant women with severe nausea and vomiting. Following sedation and pyridoxine therapy, the elevated glutamic acid level of these cases of hyperemesis gravidarum was returned to about a normal level. In subjects with benign growths, the glutamic acid level was much less than in cases of malignancy. In experiments employing albino rats, the effects of starvation, inanition, and variation of protein level in the diet, on plasma glutamic acid levels have been studied. Starvation and an increase in protein intake both appear to depress plasma glutamic acid levels.

Comparative metabolism of D-glucose-1-C^{14} and D-glucosone-1-C^{14}. Charles E. Becker* and Harry G. Day. *Dept. of Chemistry, Indiana Univ., Bloomington, Ind.*

D-Glucosone has been reported to have an 'insulin-like' effect (Hynd. *Proc. Roy. Soc. London s. B* 101: 244, 1927) and to decrease ketone body formation and prevent urinary glycosuria when given orally in human diabetes (Thannhauser and Jenke. *Arch. Exper. Path. u. Pharmakol.* 110: 300, 1925). It exists in acyclic form as a keto-aldehyde and also in cyclic form (Becker and May, *J. Am. Chem. Soc.* 71: 1491, 1949). It is stereochemically related to D-glucose and D-glucosamine. We thought that, in the intact rat, glucosone might be reduced to glucose or aminated to glucosamine. This idea was tested by giving D-glucose-1-C^{14} or D-glucosone-1-C^{14} to fasted, young adult male rats. At the end of 3–5 hours the radioactivity recovered in the exhaled CO_2, liver and muscle glycogen, and plasma or serum glucosamine was determined. The exhaled CO_2 following glucose administration was more than 3 times as radioactive as that from glucosone. The carbon skeleton of both hexoses was incorporated into liver glycogen and blood glucosamine, but decidedly larger amounts of glucosamine were synthesized from glucosone than from glucose. The distribution of the radioactivity between the exhaled CO_2, liver and muscle glycogen, and blood glucosamine suggests the existence of the following metabolic pattern:



A biologically active product of vitamin B$_{12}$. Ernest Beerstecher, Jr. *Dept. of Biochemistry, School of Dentistry, Univ. of Texas, Houston.*

Thermal treatment of crude sources of intrinsic factor produces an intrinsic factor-uncombinable thermolabile factor which stimulates the growth of bacteria in the presence of vitamin B_{12} under assay conditions which have hitherto been found to produce a response only to vitamin B_{12}. This response is considerably greater than that produced by similar amounts of the vitamin. The data suggest that this factor is a normal product of vitamin B_{12} and is probably the erythrocyte maturation factor (EMF). Since the factor is inactive in the absence of the vitamin, it appears that vitamin B_{12} has more than a single metabolic product critical for growth. The evidence indicates that intrinsic factor combines with vitamin B_{12} to produce a complex, the erythrotin of Eakin and Ternberg, (*J. Am. Chem. Soc.* 71: 3858, 1950) which is normally enzymatically degraded to form the active principle, thus explaining the inability of vitamin B_{12} synthesized in the gut to manifest a hematopoietic response. The chemical properties of the active principle are being characterized.

Effect of manganese and calcium on proteolytic activity of pancreas homogenates. Orville G. Bentley (introduced by R. M. Bethke). *Ohio Agricultural Experiment Station, Wooster.*

Fresh chick and rat pancreas homogenates split casein at a very slow rate. In an attempt to improve the rate of hydrolysis certain mineral ions were added during a pre-assay incubation period. The addition of Mn^{++} or Ca^{++} (0.001 M) resulted in a two- to three-fold increase in the proteolytic activity of the homogenates as determined by the formation of non-protein-nitrogen in a reaction mixture of casein dispersed in $NaHCO_3$ and a

borate buffer at pH 8.45. Other ions tested were Al^{+++}, Zn^{++}, Co^{++}, Mg^{++}, Ni^{++}, Na^{+}, K^{+}, Cu^{++}, Pb^{++}, Fe^{++}, and Ba^{++} but these ions were all ineffective. Direct mineral additions to the assay medium were not immediately effective but in prolonged experiments (40–72 hours) the flasks containing Mn^{++} or Ca^{++} showed the most proteolysis. Further studies were made with fully activated pancreas homogenates. When these preparations were incubated in the absence of the substrate, the proteolytic activity rapidly disappeared. By adding Mn^{++} or Ca^{++} during the pre-assay incubation the proteolytic activity of such homogenates could be protected for several hours. These results suggested similar studies with crystalline trypsin and chymotrypsin. Again Mn^{++} and Ca^{++} protected the enzymatic activity of these preparations for a short time. It would appear that the apparent stimulatory effect of Ca^{++} and Mn^{++} on the proteolytic activity of pancreas homogenates might be accounted for on the basis that these ions retard the self-digestion of pancreatic enzymes during the pre-assay incubation period.

Pathway of formate in the in vitro synthesis of the methionine methyl group. P. Berg (introduced by W. Sakami). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Sakami and Welch (*J. Biol. Chem.* 187: 379, 1950) have demonstrated the ability of the intact rat and surviving rat liver slices to form labile methyl groups from formate. du Vigneaud *et al.* (*J. Am. Chem. Soc.* 72: 2819, 1950) have also shown the incorporation of the carbon of formaldehyde into the methyl groups of choline. The mechanism of the synthesis of methionine methyl groups from formate has now been studied in guinea pig liver slices. Using this system it has been possible to show that methionine methyl groups can be formed from formate by a pathway not involving the methyl groups of choline or betaine. This has been accomplished by demonstrating that the formation of methionine from choline and homocysteine can be inhibited without affecting the formation of methionine from formate and homocysteine. C^{14}-labeled formate and non-labeled homocysteine were incubated with liver slices, both in the presence and absence of added unlabeled pools of choline and betaine. The dilutions of radioactivity incorporated into the methyl group of methionine were such, as to exclude these compounds, as such, as intermediates in the formation of methionine from formate. Similar studies have indicated that formaldehyde *per se* is not an intermediate in the synthesis of the methyl group of methionine from formate in this system.

Coenzyme of calf spleen β-glucuronidase. Peter Bernfeld,* Armand J. Guarino* and William H. Fishman. *Tufts College Med. School, Boston, Mass.*

Calf spleen β-glucuronidase has been shown to contain a coenzyme (*Science* 112: 653, 1950). It dissociates on dilution, accompanied by a decrease of activity. Dilute solutions of electrophoretically homogenous glucuronidase are markedly activated by desoxyribonucleic acid (DNA), and DNA behaves as if it were the coenzyme. Acid or alkaline hydrolysis of DNA affects only slightly its coenzyme-like properties. An acid hydrolysate (NH_2SO_4 for 20 minutes at 100°) of commercial DNA (Krishell) was fractionated by ion exchange chromatography, according to W. E. Cohn (*J. Am. Chem. Soc.* 72: 1471, 1950). Elution with increasing concentrations of HCl yielded 107 fractions of 50 ml. each. A total of at least 7 different components, as measured by the optical density at 260 mμ showed up in the eluates. Fraction 92 exhibited the highest coenzyme-like potency. Its absorption spectrum between 245 and 290 mμ, measured at pH 2.2, 5.0 and 10.2, is essentially the same as that of crystalline thymidine (kindly supplied by Dr. L. A. Manson). P content and optical density at 266 mμ, the maximum, indicate that phosphate and thymidine components occur in fraction 92 in a mole-ratio of 1.2:1. The ratio of primary to secondary phosphate was found by electrotitration to be 1:1, indicating the presence of a mononucleotide. Evidently, fraction 92 contains thymidylic acid which is probably responsible for its coenzyme-like action. It is not known, however, whether thymidylic acid is the actual coenzyme of calf spleen β-glucuronidase.

Renal function during and after diabetic coma. Lionel M. Bernstein and Edmund F. Foley (introduced by William S. Hoffman). *Hektoen Inst. for Med. Research, Cook County Hosp., Chicago, Ill.*

Specific renal function tests, including mannitol and *p*-aminohippurate clearances and T_{mPAH}, as well as urea and electrolyte clearances were run during diabetic coma in 6 patients, and repeated 1–3 additional times in each at weekly intervals following recovery from acidosis. During diabetic coma, depending upon the severity of dehydration, there was mild to severe decrease in filtration rates and effective renal plasma flows, the mechanism of prerenal azotemia. Moderate to extremely severe tubular ischemia was present during coma. Those patients recovering from the severe dehydration and acidosis without renal tubular damage later exhibited supernormal function. Filtration rates, effective renal plasma flows and T_{mPAH} reached 150–200 % of normal. This indicates that either T_{mPAH}, a functional test, does

not have a strict morphological equivalence, or that normally not all of the available renal tissue is functioning. The concomitant increase in filtration rate and effective renal plasma flow supports the latter explanation. In 2 of the 6 cases, lower nephron nephrosis resulted from prolonged, severe ischemia during acidosis. Serial clearances and serum urea course were typical for lower nephron nephrosis. In both cases, however, there was no severe oliguria. During diabetic acidosis, the excretion of large amounts of potassium is facilitated by the acidosis and the osmotic diuretic effect of glycosuria. Evidence of tubular secretion of potassium was found during coma in one case.

Enzymatic conjugation of *m*-aminophenol. SHELDON BERNSTEIN AND R. W. MCGILVERY (introduced by PHILIP P. COHEN). *Dept. of Physiological Chemistry, Med. School, Univ. of Wisconsin, Madison.*

The syntheses of the sulfate and glucuronide of *m*-aminophenol have been studied in rat liver preparations. A modification of the Bratton and Marshall method for aromatic amines has been developed in which the sulfate and glucuronide are equally chromogenic, and the free phenol is virtually unreactive. The studies have been carried out using a typical medium for reactions dependent on aerobic phosphorylation, *i.e.*, it contains fumarate, Mg^{++}, K^{+}, cytochrome *c*, and phosphate buffer, in addition to *m*-aminophenol. The synthesis of the sulfate is at least thrice that of the glucuronide, when inorganic sulfate and potassium glucuronate, respectively, are used as substrates. The evidence suggests, in conformity with the results of previous investigators, that the glucuronide moiety may be formed from three carbon acids, since we have found lactate, pyruvate and phosphoglycerate to be as effective as glucuronate. On the other hand, the hexose phosphates are inert unless a muscle fraction rich in aldolase is added. This is in accord with the observations of others on an apparent defficiency of aldolase in rat liver. Other properties of the enzymatic system will be described. (Aided by grants from the American Cancer Society and the Wisconsin Alumni Research Foundation.)

Nature of enzymes responsible for hydrolysis of glutathione. FRANCIS BINKLEY AND C. K. OLSON.* *Univ. of Utah College of Medicine, Salt Lake City.*

The enzymes of pig kidney responsible for the hydrolysis of glutathione were not destroyed by prolonged digestion with proteolytic enzymes. The enzyme responsible for the hydrolysis of cysteinylglycine was found in preparations of pentosenucleic acid which appeared to be free of protein; these preparations were found to hydrolyze a variety of dipeptides (glycylglycine, leucineamide, etc.). Slightly modified conventional methods were satisfactory in the preparation of highly active pentosenucleic acids and a variety of procedures designed to remove protein were without effect upon the activity. The enzyme responsible for the hydrolysis of glutathione to cysteinylglycine (glutathionase) was concentrated by methods for the preparation of desoxyribonucleoproteins followed by the removal of protein by digestion but highly purified preparations of desoxyribonucleic acid were found to be inactive. The most highly purified active materials contained considerable amounts of lipid and were obtained from digested preparations of desoxyribonucleoproteins by flotation during centrifugation. The preparations of pentosenucleic acid were inactive toward dipeptides in the absence of added divalent metal ions but, when preparations of the glutathionase were added to reconstitute the complete system for the hydrolysis of glutathione, metal ions were no longer required for the hydrolysis of dipeptides.

A heat stable co-factor for glutathione synthesis. KONRAD BLOCH. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

The enzyme system from pigeon liver which catalyses the synthesis of glutatione from the constituent amino acids of the tripeptide in the presence of phosphate, Mg^{++} and adenosine triphosphate (JOHNSTON AND BLOCH. *J. Biol. Chem.* January, 1951) is stable when kept at −20°C, but is rapidly inactivated at O°C. Standing at O°C, for 6 hours reduces enzymatic activity to about one-fifth the original. Activity of the aged system is fully restored by addition of a boiled water extract prepared either from pigeon liver acetone powder or from acetone-dried yeast. The co-factor effect was not shown by any of the following compounds: adenylic acid, adenosine triphosphate, glutamine, DPN, TPN, vitamin B_{12}, pteroyltriglutamate, pyridoxalphosphate or coenzyme A. Coenzyme A in low concentrations, markedly depressed the rate of glutathione synthesis. Five different preparations of this co-factor ranging in potency from 12–170 U per mg. caused an inhibition which was roughly proportional to their coenzyme A content. Incubation of the liver enzyme system at 37° for 30 minutes results in nearly complete loss of activity. In this case the addition of the heat stable co-factor affords only partial reactivation. Requirements for the co-factor can also be demonstrated for the synthesis of glutathione from glutamyl-cysteine and glycine.

Effects of N-allyl normorphine on cholinesterases. THOMAS R. BLOHM AND WANDA GONZALEZ WILLMORE (introduced by ERNEST BEERSTECHER).

Dept. of Biochemistry, Univ. of Texas School of Dentistry, Houston, Texas.

The effects of N-allyl normorphine on the hydrolysis of acetylcholine by cholinesterases of brain, red cells and plasma were measured manometrically and compared to the effects of morphine. N-allyl normorphine is a slightly more potent inhibitor of all types of enzyme than is morphine in equivalent molar concentration.

Excretion of radioactive 17-methylestradiol. BERNADETTE C. BOCKLAGE,* E. A. DOISY, JR.,* WILLIAM H. ELLIOTT,* S. A. THAYER, HAROLD J. NICHOLAS* AND EDWARD A. DOISY. *Depts. of Biochemistry and Internal Medicine, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Since other workers have demonstrated the importance of the liver in estrogen metabolism, studies were undertaken to ascertain the fate of orally administered 17-methylestradiol. The estrogenic activity of this compound in rats was found to be 3300 U/mg. by subcutaneous injection and 100 U/mg. by oral administration. Twenty μg. (2 oral U, 13,135 c/m) of radio-17-methylestradiol was administered by stomach tube to adult spayed female rats with bile fistulas or with ligated bile ducts immediately post-operatively. Low fecal radioactivity indicated almost complete absorption, even after the secretion of bile into the duodenum had ceased. Absorption and excretion into the bile occurred rapidly with approximately 10% excretion into the bile occurred rapidly with approximately 10% excreted during the first 4 hours. Seventy % of the radioactivity was excreted in the bile during the first two days, smaller amounts on the third day and none on the fourth day. The duct-ligated animals excreted the major portion of radioactivity in the urine at a slower rate, indicating that the urinary route may substitute for the main pathway, the biliary tract, when the latter is obstructed.

Influence of an adrenal steroid, Reichstein's Compound L, 3 β-acetoxy-17α-hydroxyallopregnan-20-one, on levels of vitamin A in plasma, liver and kidney of the rat. OSCAR BODANSKY AND BLANCH MARKARDT.* *Memorial Center for Cancer and Allied Diseases, New York City.*

Repeated administration of Reichstein's Compound L to 4-month-old rats was found to produce a marked decrease in the level of plasma vitamin A. Effect of daily administration of Compound L on development of avitaminosis and growth in groups of weanling rats receiving different amounts of vitamin A daily for 5½ weeks was then studied. At the end of this period, the concentrations of plasma vitamin A were found to be significantly lower in the L-injected groups than in control groups at comparable vitamin A intakes. Maximal blood levels were attained at a daily intake of about 25 IU and were 37 IU and 89 IU per 100 cc. in the L-injected and control series, respectively. With increasing vitamin A intake, the concentration in the kidneys of control groups rose from 1.2 IU to 20.5 IU per gm., then paradoxically decreased. In the L-injected animals, these changes were suppressed, the kidney vitamin A remaining essentially the same at 1–3 IU per gm. Neither the controls nor the L-injected animals showed any liver vitamin A storage at daily intakes of zero or 2 IU, but as daily intake was increased up to 50 IU, the livers showed marked increases in storage which was significantly greater in the L-injected animals than in controls. Results indicate that Compound L alters equilibrium governing release of vitamin A from liver.

Evidence for the formation of an intermediate in the metabolism of 3-hydroxyanthranilic acid. ANN H. BOKMAN* AND B. S. SCHWEIGERT. *Div. Biochemistry and Nutrition, American Meat Inst. Fndn., and Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Spectrophotometric evidence has been obtained for the formation of a compound other than quinolinic acid, resulting from the metabolism of 3-hydroxyanthranilic acid by extracts of acetone powders of rat liver. The absorption spectrum of 3-hydroxyanthranilic acid in phosphate buffer, pH 7.4, was found to have a maximum between 315 and 320 mμ, as determined in the Beckman spectrophotometer. Addition of extracts of acetone powders of rat liver to the Beckman cell containing the substrate resulted in a rapid rise in optical density from the initial value for 3-hydroxyanthranilic acid to a maximum after 3–4 minutes, with a subsequent slow decrease to almost blank values after one hour. This indicated the formation from 3-hydroxyanthranilic acid of another compound, which absorbed in the same region with a maximum at 360 mμ, and with an extinction coefficient greater than that of 3-hydroxyanthranilic acid. If the incubations were carried out in a 37° water bath for 1–10 minutes, and the reaction stopped by addition of either trichloracetic or perchloric acid, spectral analysis of the acid filtrates showed an absorption maximum of 315 mμ in contrast to the maxima at 298 and 268 mμ found for 3-hydroxyanthranilic acid and quinolinic acid respectively, in acid solution. As incubation times were increased beyond 10 minutes, a decrease in the absorption of the acid filtrates at 315 mμ occurred with a corresponding increase in quinolinic acid produced as determined spectrophotometrically and microbiologically.

Effect of various hormones on content and conversion of radioactive iodine (I^{131}) in the rat

thyroid. ALBERT L. BOTKIN* AND H. JENSEN. *Army Med. Service Field Research Lab., Fort Knox, Ky.*

Tracer doses of I^{131} (5 μc. without carrier) were injected intraperitoneally 2 hours before sacrifice and thyroids analyzed for total and organic I^{131} (percentage of injected dose). Administration of 0.10 mg. epinephrine simultaneously with the I^{131} produced a 56% depression of the total I^{131} content as compared to control values. Adrenal cortex extract, cortisone, and adrenocorticotrophic hormone all failed to give any significant alteration of the I^{131} content. Animals receiving identical therapy to the three preceding groups and 0.10 mg. epinephrine in addition showed only a cumulative depression effect of the two treatments. Thyroxine caused a depression of 80% in the I^{131} content which was not noticeably affected by a concurrent administration of 0.10 mg. epinephrine. The effects of thyrotrophic hormone on I^{131} content produced the following results compared with control values: 38% decrease when hormone was given 3 hours before sacrifice, 8% decrease when given 12 hours before sacrifice, no significant decrease (about 4%) when given 19 hours before sacrifice, and 25% decrease when given 24 hours before sacrifice. In all the experiments reported, the ratio of organic bound to the total I^{131} content of the glands was significantly altered only in those animals receiving thyroxine, in which groups there was a 20% depression in this ratio. The interpretation and significance of these findings as well as correlation with findings of the protein-bound iodine (I^{131}) of serum obtained under similar circumstances will be discussed.

Concentration of potassium in glomerular fluid of the Necturus. PHYLLIS A. BOTT. *Dept. of Physiological Chemistry, Woman's Med. College of Pennsylvania, Philadelphia.*

Serum or heparinized plasma and glomerular fluid collected by the method of Wearn and Richards (*Am. J. Physiol.* 71: 209, 1924) were analyzed by a new ultramicro modification of the cobaltinitrite method (KRAMER AND TISDALL. *J. Biol. Chem.* 46: 339, 1921. HOFFMAN, *J. Biol. Chem.* 120: 57, 1937). Samples 0.2 cu. mm. in volume, with potassium content of the order of 0.02–0.04 μg. were mixed with the reagent in hairpin-shaped tubes, the rounded parts of which were made of plastic and one of the glass arms of which was shortened. Excess reagent was expelled from the resulting precipitate by gentle air pressure on the long arm while filter paper was held against the short arm. Wash fluids were introduced into the short arm and finally expelled in the manner described. A reddish-purple color, developed by the dissolved precipitate in a volume of 1.2 ml. with benzidene and alpha naphthylamine, was read photoelectrically. Results compare well with those of flame photometer determinations using 0.1 ml. samples of serum. The concentration of potassium in glomerular fluid of Necturi was found to be within experimental error of that of serum or heparinized plasma if the cells were separated immediately. The method described appears to be suitable for analysis of tubule fluid.

Proteolytic, hypotensive and thromboplastic properties of hypochlorite-treated trypsin. DONALD E. BOWMAN. *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis.*

In earlier reports it was pointed out that the hypotensive and proteolytic activities of trypsin are separable. This was demonstrated with the aid of the various bean trypsin inhibitors, by iodination of trypsin, and other means. In the present study it has been found that partial chlorination and oxidation of trypsin by treatment with hypochlorite serves to reduce the hypotensive activity of the enzyme even more specifically than does iodination. Treatment with hypochlorite reduces the hypotensive activity of trypsin to less than one-tenth of its original level without diminishing its proteolytic activity. Danger of intravascular coagulation following injection of chlorinated trypsin is comparable to that associated with the untreated enzyme in rabbits; however, embolism is eliminated when heparin is injected prior to or with chlorinated trypsin. The proteolytic activity of chlorinated trypsin is inhibited by the various bean trypsin inhibitors which we have described previously; however, it is less subject to the inhibiting influence of plasma than is untreated trypsin and elevates the proteolytic activity of plasma for several hours following a single injection. Fatal embolism in rabbits is greatly diminished in the absence of heparin if the bean trypsin inhibitors are added to chlorinated trypsin prior to intravenous administration. However, the protease thromboplastic activity does not necessarily parallel proteolytic activity suggesting that these characteristics of trypsin-like proteases may also be separable. The possible advantages of comparatively shock-free chlorinated trypsin with heparin for the digestion of undesirable protein deposits including tuberculous caseous material are being investigated.

Comparison of seven methods for determination of total protein in serum and plasma. RUSSELL O. BOWMAN AND D. E. HOUSHOLDER (introduced by EDWARD MUNTWYLER). *Dept. of Biochemistry, Baylor Univ. College of Medicine, Houston, Tex.*

Pooled samples of normal serum, solutions of bovine and human fractions I, II and V, and abnormal sera were analyzed in replicate by gravi-

metric, tyrosine, nitrogen, ultraviolet absorption at 280 mμ, biuret, sulfosalicylic and trichloracetic acid procedures. The other results were compared with the gravimetric determination, or weight for the plasma fractions. Tyrosine color decreased with aging of the samples. Trichloracetic acid turbidities were not reproducible even when gum ghatti was added. The biuret procedure was variable on replication. The nitrogen and sulfosalicylic acid methods gave low values with high-globulin sera, while the ultraviolet absorption method gave high values with these. Fraction V was overestimated with sulfosalicylic acid, underestimated with the ultraviolet procedure. Fraction II was underestimated with sulfosalicylic acid and overestimated with ultraviolet. The significance of these findings and their applications to clinical analyses will be discussed.

Vitamin B_{12} and cyanide metabolism. G. E. Boxer, J. C. Rickards,* C. Rosenblum* and D. T. Woodbury.* *Research Labs., Merck and Company, Inc., Rahway, N. J.*

Methods have been developed which permit the colorimetric determination and isolation, in a form suitable for isotope analysis, of millimicrogram quantities of cyanide. The following cyanide fractions can be quantitatively separated from urine and tissue homogenates: *1*) free (or loosely found) cyanide, *2*) cyanide liberated from vitamin B_{12} by illumination, *3*) cyanide obtained by oxidation of thiocyanate (Boxer and Rickards. *Arch. Biochem.* In press). After injection of 500γ $C^{14}N^-$ (sp. act. 1 mc/mM) into a 9-kg. dog, about 30% of the radioactivity appeared in the urine over a period of 7 days. About half of this activity (15%) was present as thiocyanate representing the established detoxification mechanism for cyanide. The free (or loosely bound) cyanide excreted in the urine during the first 24 hours was of low specific activity indicating at least a 100-fold dilution in a rapidly turning over metabolic pool of cyanide. The cyanide bound in the vitamin B_{12} excreted in the first 24 hours had a specific activity about 12 times as high as the free cyanide and 4 times as high as the thiocyanate. This indication of a metabolic activity of the cyano group of B_{12}, independent from the rest of the molecule, was further emphasized by the fact that on the following day the specific activity of the B_{12} cyanide had fallen sharply, apparently through interaction with the cyanide pool of low specific activity. A small part of the activity in the urine was found in allantoin.

Combination of toluidine blue with frog sperm resulting in chromosome damage. Robert Briggs (introduced by J. A. Stekol). *Inst. for Cancer Research and Lankenau Hosp. Research Inst., Philadelphia, Pa.*

Following treatment with toluidine blue, sperm cells of *Rana pipiens* retain their motility and capacity to activate eggs. However, the eggs develop as typical haploids (with half the normal number of chromosomes), showing that the sperm chromosomes are inactivated by the dye, presumably through combination with the chromosomal DNA. In order to produce this inactivation sperm suspensions (20,000–40,000 cells per cu. mm.) are exposed to 5×10^{-6} M dye in 10% Ringer's solution for 10–20 minutes at 18°C. Among other dyes tested, methylene blue is about as effective as toluidine blue. Thionin and acriflavine, while effective as specific sperm nucleus inactivators, are less powerful in this respect—requiring longer exposures or higher concentrations. Methyl green appears to be completely without effect on the living sperm nucleus. The dye-sperm system can be exploited for the purpose of obtaining quantitative biochemical information concerning chromosome function. Dye concentration and sperm number can be easily measured, and the functional capacity of the treated sperm chromosomes can be determined by observing the development of eggs inseminated with the treated sperm. The first step in studies of this type is to determine the amount of dye which must be taken up by the sperm in order to produce complete inactivation of the sperm chromosomes. Our measurements indicate that the amount of toluidine blue taken up per sperm cell is about 10^{-16} mole. Techniques of measurement and significance of results will be discussed.

Effect of pyridoxine deficiency upon respiration and transaminase activity of cardiac muscle in ducklings. Myron Brin and Robert E. Olson (introduced by H. C. Trimble). *Dept. of Nutrition, Harvard School of Public Health and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Pyridoxine deficiency was induced in young ducklings by restriction of the vitamin from a purified diet. When anemia was evident, the deficient birds were sacrificed for respiration studies and assays of transaminase activity of heart ventricle *in vitro*. The rates of oxygen consumption, and pyruvate and lactate utilization by slices of deficient cardiac muscle were not significantly different from those of pair-fed controls. Rates of transamination in both slices and homogenates, however, were depressed. Assays for activity of both the glutamic-aspartic and the glutamic-alanine systems were carried out in homogenates of deficient and normal birds in phosphate-saline buffer at pH 7.4. Activity of the glutamic-aspartic system was measured by the method of S. R. Ames and C. A. Elvejhem. (*J. Biol. Chem.* 166: 81, 1946). In the assay of the glutamic-alanine system, homogenate was incu-

bated with glutamate and pyruvate for 20 minutes. The alanine formed was then determined by treatment with ninhydrin and estimation of the acetaldehyde colorimetrically. In pyridoxine deficiency in ducks it was found that the activity of the glutamic-aspartate system was depressed 30% and that of the glutamic-alanine system 45% below normal. Restoration of this depressed activity to near normal values could be achieved by parenteral treatment of the ducks 16 hours prior to sacrifice with 10 mg. of either pyridoxine, pyridoxamine or pyridoxal or by supplementation of the homogenate *in vitro* with 10 μg. pyridoxal phosphate.

Comparative biological activity of crystalline citrovorum factor and pteroylglutamic acid. HARRY P. BROQUIST,* E. L. R. STOKSTAD AND THOMAS H. JUKES. *Lederle Labs. Div., American Cyanamid Company, Pearl River, N. Y.*

A crystalline substance with the properties of the 'citrovorum factor' (CF) was prepared from pteroylglutamic acid (PGA) (BROCKMAN *et al. J. Am. Chem. Soc.* 72: 4325, 1950). Expressed as the anhydrous free acid, the amounts of this crystalline substance, 'leucovorin,' required for half-maximum growth per ml. of PGA-deficient medium were 0.00015 μg. for *Leuconostoc citrovorum* 8081, 0.00037 μg. for *Streptococcus faecalis* R, and 0.00017 μg. for *Lactobacillus casei* 7469. Injection 3 times weekly with 9 μg. of leucovorin gave about 60% of the maximal growth and about 88% of the maximal hematopoietic response with chicks on a PGA-deficient diet. However, when leucovorin was administered by pipette or by incorporation in the diet a response quantitatively poor or variable by comparison with PGA was obtained. Leucovorin was about 8 times as effective as PGA in reversing the toxicity of 4-amino PGA for growth of *S. faecalis*. The toxic effect of 4-amino PGA for mice was reversed by injecting simultaneously an approximately equal amount of leucovorin, but this amount was ineffective if withheld for one hour after the injection of the antagonist. Under the latter conditions the toxicity of 4-amino PGA was alleviated by injecting much greater amounts of leucovorin. Fresh natural materials including broccoli, kale, spinach, and rat liver were examined by various methods for folic acid and CF. The folic acid activity of these materials could be only partially accounted for as CF, indicating that both PGA and CF occur in nature.

Distribution of water, electrolytes, nitrogen and chondroitin sulfate in hyaline cartilages. THOMAS D. BROWER,* LILLIAN EICHELBERGER AND MICHAEL ROMA.* *Div. of Orthopedic Surgery, Univ. of Chicago, Chicago, Ill.*

Gross chemical data on hyaline cartilages will be presented and will be utilized to furnish a histochemical description of these tissues, first, per 100 gm. of cartilage solids and secondly, per kilo of fresh cartilage. *1)* 100 gm. of hyaline cartilage solids are composed of an extracellular mass represented by the weight of connective tissue solids plus the weight of the chondroitin sulfate phase and an intracellular mass. In trachea solids: extracellular mass, $(E)_s$ = 80 gm. $\sigma 3$; intracellular mass $(C)_s$ = 20 gm. $\sigma 3$; in nasal septa, $(E)_s$ = 75, $\sigma 2$; $(C)_s$ = 25, $\sigma 2$; in costal solids, $(E)_s$ = 74, $\sigma 3$; $(C)_s$ = 26, $\sigma 3$; in articular solids, $(E)_s$ = 68, $\sigma 2$; $(C)_s$ = 32, $\sigma 2$. *2)* One kg. of fresh cartilage: articular; extracellular phase $(E)_T$ = 662 gm. $\sigma 42$, of which 222 gm. are the solids and 440 gm. the water and an intracellular phase $(C)_T$ = 338 gm. $\sigma 42$, of which 74 gm. are the solids and 264 gm. the water; Costal; $(E)_T$ = 602 gm. $\sigma 49$ of which 188 gm. are the solids and 414 gm. the water and an intracellular phase $(C)_T$ = 398 gm. $\sigma 49$, of which 66 gm. are the solids and 332 gm. the water.

Metabolism of some amino acid analogs. EDWARD C. BUBL* AND JOSEPH S. BUTTS. *Dept. of Agricultural Chemistry, Oregon Agricultural Experiment Station, Corvallis.*

D-Serine has been shown to inhibit the growth of a number of micro-organisms and is also known to cause injury and death to rats. D-Glucosamic acid (chitosamic acid) has the same steriochemical configuration as D-serine. This acid was prepared by the method of Pringsheim which does not produce any change in the configuration of the 2 through 5 carbon atoms. Testing of this compound on various micro-organisms indicated a high inhibition of growth on some of these organisms. Variable results were obtained from a study of the effects of this compound on the protein metabolism of growing rats. Phenyllactic acid is a normal metabolic product of phenylalanine in some organisms and higher animals. Phenyllactic acid and a large number of ring substituted phenyllactic acids were prepared by a new method. The effects of these compounds on the metabolism of both micro-organisms and rats were determined. Depending on the type of ring substitution these compounds were found to be growth inhibitors or growth stimulators to various micro-organisms. DL-Phenyllactic acid was found to be capable of replacing phenylalanine in the nutrition of the rat. Similarly DL *p*-hydroxyphenyllactic acid is capable of replacing all of the tyrosine in a diet suboptimal in phenylalanine.

Blood-group-specific substances found in human meconium. DOROTHY J. BUCHANAN* AND S. RAPOPORT. *Children's Hosp. Research Fndn., Cincinnati, Ohio.*

Meconium, the very characteristic first stool of a newborn infant, was found to contain large

amounts of a polysaccharide, which possessed blood-group-specific activity as demonstrated by its ability to inhibit isoagglutination. (*Science* 112: 150–153, 1950). In 51 of 53 infants, the activity demonstrated by the polysaccharides in the meconium was found to correspond with the blood type of the individual baby and his ability to secrete blood-group-specific substances in his saliva. In two cases of type O infants, O activity was demonstrated in the meconium but no trace of O activity could be found in the saliva. Meconiums possessing type A or B substances were considerably more active than meconiums possessing AB or O activity. A chemical study of representative samples of crude meconium possessing the different types of activity and of the polysaccharides obtained from the meconiums by a Sevag purification procedure indicated that the blood-group-specific substances composed approximately 80% of the original meconium. No major differences in the composition of the polysaccharides possessing A, B, AB, or O activity, or those from non-secretors could be demonstrated. An analysis on the meconium from a calf fetus indicated that the polysaccharide content was lower than that of human meconium. A purified sample of the calf meconium resembled the human polysaccharides closely, but had no human blood group A or B activity.

Minerals in normal and atherosclerotic aortas. R. C. Buck* and R. J. Rossiter. *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*

The concentration of non-lipid phosphorus, calcium, magnesium, water and fat-free dry residue was determined in a series of normal and atherosclerotic human aortas. In aortas that appeared normal on macroscopic examination there was an increase in the concentration of non-lipid phosphorus, calcium and magnesium with age. The rate of increase was, in each case, logarithmic. This is in contrast to the findings for lipids (*Federation Proc.* 9: 156, 1950). The concentration of each of the minerals in atherosclerotic portions of aorta was significantly greater than in normal portions, but the increase associated with atherosclerosis was small in comparison with that associated with ageing. The concentration of water was significantly decreased in relation to the degree of atherosclerosis, but that of the fat-free dry residue was unchanged. The relative proportions in which the minerals, non-lipid phosphorus, calcium and magnesium, are deposited was constant, suggesting the possibility that they are in chemical combination. The ratio of calcium to phosphorus was similar to that found in bone.

Metabolic products of bacteria-free *Ascaris lumbricoides*. Ernest Bueding and Helen W. Yale.* *Dept. of Pharmacology, Western Reserve Univ., Cleveland, Ohio.*

According to Weinland (*Ztschr. Biol.* 45: 113, 1904) valeric rather than lactic acid is the major product of fermentation of *Ascaris*. However, this parasite harbors a great variety of bacteria, some of which synthesize valeric acid. Since formation of this acid is uncommon for metazoa, the observed valeric acid production may have been due to metabolic activities of contaminating bacteria. Furthermore, we have observed that, in the presence of ATP, aqueous extracts of acetone powders of *Ascaris* muscle produce lactate, but no volatile acids. Attempts to investigate this question were made by using *Ascaris* previously sterilized by treatment with antibiotics. Media in which such organisms had been incubated contained considerable amounts of volatile acids (1.2–1.8 mEq. produced by 100 gm. of worms in 24 hours). They were separated on buffered Celite columns with chloroform-butanol mixtures. Approximately 20% of the volatile acids contained six carbons, 40% five, 2 to 5% four, 10 to 15% three and 15 to 25% two. No formic acid production was detectable. Isomers of valeric acid were separated partially on Celite columns (non-mobile phase: 2 M phosphate buffer, pH: 7.8; mobile phase: 1% butanol in chloroform). Slightly more than 20% of the volatile acids produced by bacteria-free *Ascaris* had the solubility characteristics of n-valeric acid and of an α,β-unsaturated volatile acid; slightly smaller amounts (15–18%) had those of methylethylacetic and isovaleric acid. (This work has been supported by a grant from the U. S. Public Health Service.)

Agglutination of virus particles and bacterial cells by synthetic polypeptides. Warren C. Burger and Mark A. Stahmann (introduced by Robert H. Burris). *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

High molecular weight synthetic lysine polypeptides were found to agglutinate and precipitate approximately fourteen times their weight of tobacco mosaic virus. The polypeptides combined with the virus particles above pH 4 to cause precipitation and inhibition of virus infectivity at concentrations as low as 7 μg. polypeptide per ml. The inhibition was a logarithmic function of the polypeptide concentration and chain length. Electron micrographs showed virus aggregation at levels below those required for precipitation. Heavy bacterial suspensions were completely agglutinated by the polypeptides at concentrations greater than 100 μg. of polypeptide per ml. This agglutination, which did not occur in the absence of electrolytes, involved a combination of the

polypeptides with the bacterial cells. The data are consistent with the theory that many ionic bonds are formed between the basic peptide and acidic groups at the surface of the virus or bacteria. The inactivity of the free amino acid shows that the peptide structure is essential. The lysine polypeptide chain produces a very high local concentration of positively charged amino groups which, by mass action, favors multiple ionic bond formation between polypeptide and the virus or bacteria. Since the probability that all the ionic bonds with a polypeptide molecule would be broken at the same instant is low, the resulting complex is stabilized. The multipolar character of the polypeptides then allows formation of network type structures leading to agglutination and precipitation.

One-quantum mechanism and energy cycle in photosynthesis. DEAN BURK AND OTTO WARBURG.* *Kaiser-Wilhelm-Institut für Zellphysiologie, Berlin-Dahlem.*

If one illuminates Chlorella at pH 4–5 intermittently with a high intensity of monochromatic light and equal light and dark periods of the order of a minute, a minimum quantum requirement of *1* is observed in each light minute, that is, one mole of abosrbed light quanta effects the absorption of one mole of CO_2 and production of about the same quantity of O_2 ($-CO_2/+O_2 = -1$ to -1.2). This 'quantum yield' of 1 is obtainable with either red, green, or blue light (λ = 644, 547, or 436 mμ). In each dark-minute may be observed a combustion process ($+CO_2/-O_2 = -1$ to -1.2) whose rate increases, as the light intensity in the illuminated periods is increased, to over 10 times the rate of the ordinary dark respiration. In this combustion phase, which also proceeds in the light-periods, some two-thirds or more of the organic product formed in the photochemical phase is immediately burned to yield with high efficiency the chemical energy necessary to form a photosynthetic substrate wherewith one quantum only is needed to effect consumption of one molecule of CO_2 and production of one molecule of O_2. Photosynthesis thus takes place by means of a two-phase cycle of interdependent energy transformations, one photochemical, the other chemosynthetic. This energy cycle is the solution of the long-standing quantum problem in photosynthesis, namely, how a chemical reaction that thermodynamically requires the energy-equivalent of several light quanta is accomplished. Three to four revolutions of the photosynthetic energy cycle have been found to suffice to yield a permanent gain of one molecule of CO_2 fixed and O_2 produced. (Cf. *Naturwiss.*, Dec., 1950; *Ztschr. Naturforsch.*, Feb.–March, 1951).

Separation of lactic, succinic and malonic acids by ion-exchange chromatography. HARRIS BUSCH,* ROBERT B. HURLBERT* AND VAN R. POTTER. *McArdle Memorial Lab., Med. School, Univ. of Wisconsin, Madison.*

Difficulties encountered in metabolic studies involving the separation of succinic and lactic acids by silica gel chromatography led to the use of anion exchange columns. Satisfactory separation was achieved by elution from Dowex-1 (formate form) with increasing concentrations of formic acid. The separated non-volatile acids were then determined by titration after the volatile eluant was removed by vacuum desiccation over sodium hydroxide. The neutralized acids (30–100 μM) were mounted on a 200–300 mesh resin column 10.5 cm. high and 1.0 cm. in diameter, packed with about one pound of air pressure. The concentration of the formic acid eluant was smoothly increased by forcing 4 *N* formic acid from a large reservoir through a 200-ml. mixing flask (originally filled with distilled water and mixed with continuous magnetic stirring) and through the column by means of air pressure, according to a similar procedure developed by one of us (R.B.H.) for pyrimidine separations. The concentration of formic acid was about 2.8 *N* in tube 100 when collected on a Technicon fraction collector with 40 drops per tube and 10 drops per minute. The first few tubes were found to contain the lactic acid fraction, while succinic acid was in tubes 30–35 and malonic acid was in tubes 75–85. Further studies with related acids are in progress.

Evidence for catabolism of uric acid in normal humans. JAMES BUZARD, CHARLES BISHOP AND JOHN H. TALBOTT (introduced by EDWARD M. BRIDGE). *Depts. of Medicine and Biochemistry of Univ. of Buffalo Med. School and Buffalo General Hosp., Buffalo, N. Y.*

Two healthy male subjects were injected intravenously with N_{15} labeled uric acid on two separate occasions. The urinary excretion of uric acid was followed using the isotope dilution technique and from this the amount of the injected N_{15} uric acid excreted via the urinary route was calculated. The determinations were continued until no more labeled uric acid was being excreted. The recovery of the injected uric acid in the four experiments was: 68%, 71%, 74% and 76%. Since the pool size and the turnover rate of uric acid were known in each case, it was possible to estimate the average quantity of uric acid synthesized in the body daily. From this datum and the average daily excretion of uric acid as determined by isotope dilution, it was possible to calculate the amount of synthesized uric acid excreted as urinary uric acid. These values were 61%, 63%, 74% and 71%,

respectively, and are similar to the values above which were obtained by independent calculations. Thus it was calculated that approximately two-thirds of the uric acid synthesized by the body was excreted as urinary uric acid and the remaining one-third was disposed of by another route, presumably catabolism.

Effect of Antabuse on Evipal-induced sleeping time in rats. ELIZABETH CARMICHAEL, W. DONALD GRAHAM AND M. G. ALLMARK (introduced by L. I. PUGSLEY). *Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Ontario, Canada.*

Persky *et al.* (*J. Pharmacol. & Exper. Therap.* 100: 273, 1950) have attributed to barbiturates the ability to inhibit acetaldehyde dehydrogenase through competition with the substrate. It has been demonstrated by Graham (*J. Pharm. Pharmacol.* In press) that Antabuse inhibits this same enzyme apparently through competition with diphosphopyridine nucleotide. It has now been found that oral dosage with Antabuse, 24 hours prior to the intraperitoneal injection of Evipal, significantly prolongs the sleeping time of rats. For example, rats receiving 0.5 gm. of Antabuse per kg. body weight by stomach tube 24 hours prior to the injection of Evipal, remained comatose twice as long as rats given the same dose of Evipal alone. These results may have significance in clinical medicine in two ways: *1*) Alcoholics receiving Antabuse may be abnormally sensitive to barbiturates. *2*) In patients receiving barbiturates regularly, the coadministration of Antabuse may enable reduction of the barbiturate dosage.

Separation of urinary amino acids by ion exchange chromatography. MARY E. CARSTEN (introduced by ISIDOR GREENWALD). *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

The large amounts of salts and of nitrogenous substances in urine have made difficult analysis for amino acids. A method has been developed for the preliminary separation by ion exchange of a crude ampholyte fraction which is then submitted to precise chromatography on a cation exchange resin. The total cations and ampholytes of a 24-hour sample of urine are first adsorbed on a column of a sulfonic acid resin. The weak bases and ampholytes are eluted with ammonia. This eluate is then passed through a strongly basic anion exchange resin. This adsorbs the ampholytes and rejects the weak bases. The ampholyte fraction is then eluted with HCl. An aliquot of the eluate (0.3 mEq. amino acid) is adsorbed on, then chromatographically eluted from a column of a finely divided sulfonic acid resin with increasing concentrations of HCl. The effluent is collected in 1-ml. fractions and the amino acid concentrations determined colorimetrically with ninhydrin or with 1,2-naphthoquinone-4-sulfonate. The constituent amino acids and peptides are identified by paper chromatography or by specific reactions. The application of this procedure to normal and pathological urines, to a synthetic mixture simulating urine and to normal urine with amino acids added will be illustrated.

Vitamin B_T. II-Purification and chemical studies. HERBERT E. CARTER, P. K. BHATTACHARYYA,† KATHARINE R. WEIDMAN,* AND G. FRAENKEL.† *Depts. of Chemistry and Entomology, Univ. of Illinois, Urbana.*

Crude vitamin B_T (prepared as described in the preceding abstract) was subjected to paper chromatography. In a phenol-water system the active fraction coincided with a ninhydrin-positive spot (R_F 0.83). However, in a butanol-water system the vitamin activity was separated from ninhydrin-positive material. The crude preparation could be concentrated 5–10 times over alumina columns or by Craig distribution in a phenol-water system. The gummy products thus obtained were active at 0.75 μg/gm. of diet in the *Tenebrio* assay. From these highly active fractions, crystalline materials melting in the range 190–200°C. have been obtained. The most active fractions of B_T show no characteristic absorption peaks in the ultraviolet and give weak carbonyl absorption in the infrared. Vitamin B_T is extremely resistant to acid hydrolysis but is destroyed by treatment with strong alkali. The nature of the alkaline degradation products will be discussed.

Structure of nucleic acids. III. Kinetics of alkaline hydrolysis of pentose nucleic acid. LIEBE F. CAVALIERI AND LOUIS P. HAMMETT (introduced by GEORGE BOSWORTH BROWN). *Chemistry Lab., Columbia Univ., and Sloan-Kettering Inst. for Cancer Research, New York City.*

The kinetics of the alkaline hydrolysis of yeast pentose nucleic acid has been studied by means of a stirred flow reactor (YOUNG AND HAMMETT. *J. Am. Chem. Soc.* 72: 280, 1950). The continuous flowing system allows the direct measurement of the instantaneous rate of liberation of each mononucleotide. Hydrolysis mixtures of varying degrees of conversion were eluted from Dowex #1 chloride columns for the analysis of the individual mononucleotides. Rates were calculated from the equation, rate $= \frac{u}{V}x$, where u is the flow rate, V the volume of the reactor and x the concentration of the particular mononucleotide in the reactor at the steady state. A plot of r *vs.* V/u (which is a measure of the degree of

conversion) reveals the appearance of several maxima for each nucleotide. This proves the hydrolysis to follow a consecutive-type reaction mechanism. Of the 4 nucleotides guanylic acid is distinct in that its rate of formation appears to be zero at zero time. This is taken to mean that a significant amount of hydrolysis must occur before guanylic acid becomes available for cleavage, i.e. it probably exists in a branched net-work. The following mechanism is tentatively suggested: $N \overset{k_1}{\leftarrow} C + N \overset{k_3}{\leftarrow} A \overset{k_1}{\rightarrow} N + B \overset{k_2}{\rightarrow} N$, where N = mononucleotides, A, B, C, D— = polynucleotides.

Spectrophotometric measurements of the cytochrome components of the succinic oxidase system. BRITTON CHANCE. *Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.*

Sensitive spectrophotometric methods have been developed for the measurement of optical density changes caused by the oxidation and reduction of cytochrome components in turbid heart muscle preparations. If sodium succinate is added to aerated heart muscle preparation (prepared according to Keilin and Hartree), slight changes of optical density occur ($\sim$ 0.3% of the total absorption in the visible region) which are plotted as a function of wavelength and show the characteristic bands of cytochrome components a, b, and c at the positions 605, 562, and 552 mμ (605, 564, and 550 were measured visually by Keilin and Hartree). These absorption bands are greatly intensified some time later when the oxygen is exhausted and complete reduction of the cytochrome components by succinate occurs. The band of a_3 (445 mμ) then appears. It is concluded that the cytochrome components of heart muscle preparations are not fully oxidized in steady-state action in the presence of oxygen and succinate. The intensity of the 'steady-state oxidized' spectrum of cytochrome components a and c is reduced by succinic dehydrogenase inhibitors (malonate) and intensified by oxidase inhibitors (cyanide). On the basis of the spectroscopic measurements and on the basis of some assumptions to be discussed, the sequence of steady-state percentage oxidation for a particular heart muscle preparation is $a_3 = 95$, $a = 77$, $c = 64$, $b = 58$. In addition, the time sequence of reduction of the cytochrome components has been measured, and is found to be $c < \overset{a}{a_3} < b$ on the basis of the interval between addition of succinate and 50% reduction of each component.

Metabolism of formaldehyde by rat liver homogenates. FRIXOS CHARALAMPOUS AND GERALD C. MUELLER (introduced by JAMES A. MILLER). *McArdle Memorial Lab., Med. School, Univ. of Wisconsin, Madison.*

The ability of rat liver homogenates to utilize formaldehyde enzymatically has been studied in fortified reaction mixtures under a variety of conditions. It was observed that 40 mg. of homogenized rat liver freshly prepared in isotonic KCl and incubated in a reaction flask containing phosphate buffer, isotonic KCl and magnesium ions metabolized 60–65 of formaldehyde in 20 minutes. This activity was completely destroyed when the homogenate was incubated for 30 minutes at 38° prior to addition to the formaldehyde-containing system. Similarly, homogenates prepared in distilled water rapidly lost the ability to metabolize formaldehyde in the absence of added cofactors. This inactivation was found to be prevented or completely reversed by the addition of diphosphopyridine nucleotide; hexosediphosphate, and nicotinamide to the reaction flask. With incubated homogenates triphosphopyridine nucleotide, adenosinetriphosphate, glucose-6-phosphate and nicotinamide could not substitute for diphosphopyridine nucleotide and hexosediphosphate. The rate of enzymatic removal of formaldehyde was slightly greater in an atmosphere of nitrogen than in air. Under anaerobic conditions only 17–20% of the metabolized formaldehyde could be accounted for as formic acid and methanol combined. In the same system formic acid and methanol were not utilized. Aerobically, 27–40% of the metabolized formaldehyde was accounted for as these two metabolites, however there was also a variable utilization of these substances in air. The metabolic interrelationships of formaldehyde, other 'one carbon' compounds, and acceptor systems will be discussed.

Crystalline human carbonylhemoglobin as a standard for several colorimetric methods for hemoglobin determination. MAX E. CILCOTE AND ARTHUR E. O'DEA (introduced by WILSON D. LANGLEY). *Dept. of Biochemistry, Univ. of Buffalo School of Medicine, Buffalo, N. Y.*

Human carbonylhemoglobin as lyophilized powder from crystalline oxyhemoglobin was tested as a suitable reference standard for the colorimetric estimation of hemoglobin. Crystalline oxyhemoglobin from outdated transfusion blood was dialyzed free of salts, converted to carbonylhemoglobin, and lyophilized. The iron content was used as the primary criterion of purity. The relation between the concentration of carbonylhemoglobin and the optical densities determined in the acid, alkali, cyan hematin and cyanmethemoglobin methods revealed straight line functions in each case. However, extinction coefficients at 535 mμ of acid hematin prepared from carbonylhemoglobin powder were appreciably less than for the acid hematin from hemoglobin in blood. A distinct increase in the extinction co-

efficients of acid and alkali hematin, at 535 and 580 mμ, respectively, was observed when normal plasma was added to the carbonylhemoglobin prior to color development, but not with the other methods. The hemoglobin contents of a series of human bloods were estimated from total iron and colorimetric determinations in the above methods with reference to the extinction values obtained on carbonylhemoglobin solutions with and without added plasma. The results indicate that the lyophilized carbonylhemoglobin can serve as a practical standard for the methods studied.

Cytochrome *b*. HAROLD W. CLARK,* HAROLD A. NEUFELD* AND ELMER STOTZ. *Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Several roles for cytochrome *b*, including identity with succinic dehydrogenase, have been postulated but not clearly demonstrated because of the difficulties encountered in separating the cytochrome components. Purification of the SC factor (*Federation Proc.* 9: 160, 1950) by cholate-ammonium sulfate fractionation of pig heart extract has resulted in a clear red fraction which has absorption characteristics attributed to cytochrome *b*. In the oxidized state this compound has a prominent absorption peak at 416 mμ and a slight peak at about 525 mμ. The reduced form absorbs very strongly at 429 mμ and 561 mμ and to a lesser degree at about 529 mμ. No absorption peaks were noted at 550 mμ and 605 mμ, indicating the virtual absence of cytochromes *c* and *a*, although weak oxidase activity remained. Cytochrome *b* is partially reduced by succinate and succinic dehydrogenase. Cytochrome *b*, reduced by hydrosulfite, is completely oxidized by cytochrome *c*. In the presence of succinate, succinic dehydrogenase, cytochromes *b* and *c*, and cyanide, the *b* component is found partially reduced and the *c* component completely reduced. On differential ultracentrifugation the ratio of SC activity to cytochrome *b* remains constant, but succinic dehydrogenase activity may be separated from cytochrome *b*. It seems probable that the labile link between succinic dehydrogenase and cytochrome *c*, heretofore called the SC factor, is cytochrome *b*.

Estimation of serum lipoproteins by chemical and physical methods. GERALDINE E. COGIN* AND MARY L. PETERMANN. *Sloan-Kettering Inst., New York City.*

Human plasma proteins have been separated by method 10 of Cohn *et al.* (*J. Am. Chem. Soc.* 72: 465, 1950) into two fractions, one containing the alpha lipoprotein and the other the beta lipoprotein. The cholesterol content of the whole plasma and of each fraction has been determined. The lipoproteins of low density (less than 1.06) have been concentrated from both serum and plasma by high speed centrifugation and examined in the analytical ultracentrifuge by a modification of the flotation method of Gofman *et al.* (*Science* 111: 166, 1950). From its refractive increment, corrected for the sector shape of the cell, the amount of the low density material has been calculated. The density of every solution analyzed in the ultracentrifuge has been checked in a micropycnometer. The flotation rate determined from the position of the maximum refractive index gradient appears to be fairly constant in normal young women, while the shapes of the flotation curves are quite variable. Comparison of the results obtained by the chemical and physical methods shows that the amount of plasma beta-lipoprotein cholesterol is proportional to the concentration of serum low-density lipoprotein over a wide range. In some instances the total low-density lipoprotein may be greatly elevated, although the proportion of the S_f 10-20 components is relatively small.

Response of rats to diets high in methionine. HAROLD P. COHEN* AND CLARENCE P. BERG. *Dept. of Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City, Iowa.*

When excessive amounts of DL-methionine (1.8%) are added to the diet of young white rats, their rate of growth is retarded and hemosiderin accumulates in their spleens (VAN PILSUM AND BERG. *J. Biol. Chem.* 183: 279, 1950). Neither of these effects was alleviated by further supplementing such diets with DL-serine or glycocyamine, in amounts molecularly equivalent to the methionine. Molecularly equivalent supplements of arginine plus glycine alleviated the growth depression, though not as well as when less arginine and more glycine were supplied. The supplements did not prevent the spleen darkening in either instance. Replacement of the 1.8% of DL-methionine with a molecularly equivalent amount of DL-homocystine induced an even greater growth retardation; when the choline content of the diets was raised from 0.2–0.8%, the growth on the homocystine regimen was equal or slightly superior to that on the high methionine diet. Gross examination of the spleens of the animals fed the homocystine showed abnormal pigmentation only in the group receiving the higher level of choline. The homocysteine and the methyl fragments of the methionine molecule are therefore tentatively assumed to exert different effects, the former (or a derivative produced from it) depressing the growth, the latter inducing the spleen pigmentation.

A comparison of hydrolytic procedures for conjugated urinary corticoids. SAUL L. COHEN. *Dept. of Physiological Chemistry, Univ. of Minnesota Med. School, Minneapolis.*

The corticoid activity (formaldehydogenic) of urines without any hydrolysis and after hydrolysis with acid, buffer and with preparations of calf spleen glucuronidase were determined and compared. The urinary corticoid values are increased about 50–100% by adjusting to pH 1.0 and permitting to stand at room temperature for 1–3 days. Permitting the urine to remain at pH 1.0 for longer periods of time results in a gradual decrease in assayable corticoids. Heating urines in n/10 acetate buffer (pH 5.0) in a boiling water bath results in up to twice the yield of corticoids than that obtained after acid hydrolysis in many but not all urine specimens. Those urine specimens for which increased corticoid values are obtained by the buffer hydrolysis show optimum values after about 2 hours of heating. Hydrolysis with preparations of calf spleen glucuronidase yields maxima corticoid assays 10–30 times greater than those obtained by the pH 1.0 hydrolysis. Subsequent treatment of the buffer or enzyme hydrolyzed urines with acid may lead to a considerable loss in corticoids. These effects are interpreted as due to differences in the nature of the conjugations and of the liberated formaldehydogenic substances.

Pentose production and utilization by enzyme systems of *Escherichia coli*. SEYMOUR S. COHEN, DWIGHT B. MCNAIR SCOTT* AND MARY LANNING.* *Children's Hosp. of Philadelphia (Dept. of Pediatrics) and Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Extracts of *E. coli* grown aerobically on glucose contain 6-phosphogluconate dehydrogenase. Enzyme preparations, purified by precipitation between 0.62–0.90 saturated $(NH_4)_2SO_4$, catalysed the formation of 0.5 mole pentose phosphate and CO_2 per mole of phosphogluconate in the presence of TPN and phenazine, O_2 (0.5 mole) was consumed. After removal of phosphate, the products were separated on a Florex column. D-Arabinose was identified. Other separated pentoses behaved like D-araboketose and anenediol-pentose. Very little ribose was found. Bacteria grown adaptively on gluconate contain gluconokinase, which catalyses the formation of 6-phosphogluconate in the presence of ATP. It has been shown in growing bacteria with C_1-labeled gluconate that most of the gluconate is degraded by means of phosphogluconate dehydrogenase via pentose phosphate. Cells grown adaptively on D-arabinose contain a specific D-arabinokinase. Acid labile phosphate of ATP was transferred to D-arabinose to form an acid-stable organic phosphate in enzyme extracts. Cells grown adaptively on D-ribose contain a specific D-ribokinase. An acid-stable organic phosphate was formed from ribose, ATP, and purified ribokinase. The isolated product behaved like the unknown pentose phosphate, thought to be enediol pentose phosphate, previously observed as a product of phosphogluconate degradation (COHEN, S. S. AND D. B. MCNAIR SCOTT. *Science* III: 543, 1950). It is considered that phosphogluconate degradation leads to a compound participating in the equilibria: D-arabinose-5-PO_4 $\rightleftarrows$ trans-enediol pentose-5-PO_4 $\rightleftarrows$ D-araboketose-5-PO_4 $\rightleftarrows$ cis-enediol pentose-5-PO_4 $\rightleftarrows$ D-ribose-5-PO_4. The pathway of phosphogluconate degradation and pentose formation appears to be a major pathway of carbohydrate utilization in *E. coli*.

Isolation of 5′ nucleotides from nucleic acid digests. WALDO E. COHN AND ELLIOT VOLKIN.* *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

In the course of analysing digests of calf-liver ribonucleic acid (RNA) produced by the consecutive actions of ribonuclease and arsenate-inhibited intestinal phosphatase (KLEIN, *Ztschr. Physiol. Chem.* 218: 164, 1933), we have isolated and identified the 5′ phosphates of cytidine, adenosine, uridine and guanosine. The only other readily identifiable mononucleotide products found were the *b* forms of cytidylic and uridylic acids (COHN, *J. Am. Chem. Soc.* 72: 2811, 1950). Several as yet unidentified peaks in the ion-exchange elution sequence are being investigated. The 5′ nucleotides were identified by comparison with synthetic materials (and muscle adenylic acid) with respect to ion-exchange behavior, resistance to acid hydrolysis of the purine compounds, spectrophotometric properties of the pyrimidine pair, a purine:pentose:phosphate ratio of 1:1:1, identical hydrolytic rates with a specific 5′-nucleotidase, absence of desoxypentose. The 5′ substances were not synthesized by the same phosphatase treatment from nucleosides or nucleotides, nor did they incorporate inorganic phosphate or arsenate from the medium. It appears, therefore that they were constituents of the original RNA, for if produced by enzymatic action during hydrolysis, this must have occurred at some polynucleotide level. Failure to isolate ribose-5-phosphate from acid hydrolysates of the same material, or 5′ nucleotides from alkaline hydrolysates, lends support to Gulland's hypothesis (*J. Chem. Soc.* 1722, 1938) of phosphate migration during chemical hydrolysis from the 5′ to the 3′ position. This would account for the recurring evidence of 5′ phosphoryl linkages in native or enzymatically degraded ribonucleic acids and not in chemically degraded material.

Binding of thiazines by plasma albumin and nucleic acid. H. BRUCE COLLIER AND GORDON M. ALLENBY.* *Dept. of Biochemistry, Univ. of Alberta, Edmonton, Canada.*

Phenothiazine is a powerful anthelmintic drug, and its oxidized derivatives are active enzyme inhibitors (COLLIER AND ALLEN. *Canad. J. Research B*20: 284, 1942). Binding of thiazone and thionol by crystalline plasma albumin was investigated by the dialysis and the spectrophotometric methods, in 0.05 M phosphate of pH 7.4. Within the limited solubility range of these compounds the degree of binding was found to be rather limited, with n values of about 2, and k values of approximately 5×10^{-6}. Preliminary experiments with yeast nucleic acid (Eimer and Amend) in the same buffer indicate a marked lowering of the extinction of both thiazone and thionol, as observed spectrophotometrically.

Enzymatic reaction between diphosphopyridine nucleotide (DPN) and reduced triphosphopyridine nucleotide ($TPNH_2$). SIDNEY P. COLOWICK, NATHAN O. KAPLAN AND MARGARET M. CIOTTI.* *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Extracts of *Pseudomonas fluorescens* contain a specific isocitric dehydrogenase which reduces TPN but not DPN. However, DPN is rapidly and completely reduced by isocitrate in this system provided that a small amount of TPN is also added. It appears that the reduction of DPN is due to an enzyme which catalyzes the following reaction: $TPNH_2 + DPN \rightarrow TPN + DPNH_2$. The enzyme has been found so far only in *Pseudomonas* extracts. It can be largely freed of isocitric dehydrogenase and diaphorase activity by ammonium sulfate or acetone fractionation. The purified enzyme can be assayed *a*) indirectly, by its effect on the rate of reduction of DPN in a system containing isocitrate, isocitric dehydrogenase from pig heart, and a catalytic amount of TPN, or *b*) directly, by its effect on the rate of reduction of DPN by chemically or enzymatically prepared $TPNH_2$. In *test system a*, all of the added DPN is reduced, while in *test system b* the added DPN is only partially reduced and a large excess of DPN is required for complete oxidation of the $TPNH_2$. However, so far it has not been possible to detect the reaction in the reverse direction; i.e. there is no reduction of TPN by $DPNH_2$, either directly or in a test system containing ethyl alcohol, alcohol dehydrogenase, and catalytic amounts of DPN.

Reduction of oxidized glutathione by plant enzymes. ERIC E. CONN* AND BIRGIT VENNESLAND. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

A new enzyme, termed glutathione reductase, has been demonstrated to occur in a wide variety of plant tissues. This enzyme catalyzes the reduction of oxidized glutathione (GSSG) by reduced triphosphopyridine nucleotide (TPNH). The reduction of GSSG proceeds to completion stoichiometrically; and no reaction can be demonstrated in the reverse direction. Reduced diphosphopyridine nucleotide can not be substituted for TPNH, nor can cystine be substituted for GSSG. The enzyme is water soluble, heat labile, and can be precipitated by ammonium sulfate with little loss of total activity. Prolonged dialysis against dilute buffers leads to variable losses of activity which can not be restored by addition of boiled extracts. The properties of glutathione reductase from wheat germ have been studied in some detail. The enzyme can be assayed quantitatively by titrating the GSH formed from GSSG in the presence of catalytic amounts of TPN plus a TPN-dehydrogenase with its substrate. Glucose-6-phosphate with *Zwischenferment* is particularly useful for this purpose. *Zwischenferment* activity is present in all the plant extracts found to contain glutathione reductase (wheat germ, carrot, cabbage, spinach, potato, avocado). In crude dialyzed extracts the former enzyme generally occurs in such amounts that addition of extra *Zwischenferment* to the test systems does not increase the rate of reduction of GSSG. The two activities can, however, be separated by ammonium sulfate fractionation. The possible function of glutathione reductase in plant metabolism will be discussed.

Chemistry of urochrome. THOMAS B. COOLIDGE. *Dept. of Biochemistry and Zoller Dental Clinic, Univ. of Chicago, Chicago, Ill.*

The observation of Thudicum that urochrome can be benzoylated has been confirmed. The ether soluble product of benzoylation is not a single substance and on treatment with hot ethanolamine yields material having approximately the solubility and light absorption in the visible of the untreated material. Highly purified urochrome preparations yield on treatment with sodium perborate by the method of Schunterman and Hoffman an ether soluble pigment of very low nitrogen content. These facts and others suggest that urochrome is a mixture of pigments having identical or very closely similar quinonoid chromophoric structures but differing in the nitrogenous side chains.

Biosynthesis of histidine. MINOR J. COON AND LOUIS LEVY (introduced by D. W. WILSON). *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

The mechanism by which histidine is produced in living cells is of particular interest because it

is now recognized that this amino acid is not essential in the diet of man for the maintenance of nitrogen equilibrium (ROSE, *Federation Proc.* 8: 546, 1949). Experimental animals, on the other hand, do not synthesize histidine to an appreciable extent. We have therefore employed yeast as an organism with which to study this metabolic pathway. A culture of *Saccharomyces cerevisiae* was allowed to grow for about 10 hours on a basal medium which was supplemented with one of several highly radioactive test substances. The protein fraction from the harvested yeast was subjected to acid hydrolysis, and the histidine was separated from the resulting mixture of amino acids with the aid of buffered cation exchange resins; it was then converted to the silver complex and finally recrystallized to constant radioactivity in the form of the bis-3,4-dichlorobenzenesulfonate. Formate-C^{14}, bicarbonate-C^{14}, and lactate-2,3-C^{14} were found to be precursors of radioactive histidine, whereas acetate-2-C^{14} and glycine-1-C^{14} were relatively ineffective in this respect. Studies on the chemical degradation of the histidine samples are in progress.

Removal and distribution of P^{32} following its injection as buffered phosphate, colloidal calcium or chromium phosphate. JOHN A. D. COOPER AND JACOB R. SUKER (introduced by CHESTER J. FARMER). *Dept. of Physiological Chemistry, Northwestern Univ. Med. School, Chicago, Ill.*

P^{32} as buffered phosphate, or as colloidal calcium or chromium phosphate was injected intravenously into dogs and rats. The dose of calcium and phosphorous was 15 and 7 mg/kg. body weight, respectively. Disappearance of excess phosphate from the blood was complete two hours after phosphate injection. The greatest localization of P^{32} occurred in the liver and kidney with lesser concentrations in the lung and spleen. Following injection of labeled colloidal calcium phosphate solution calcium disappeared from the circulation much more rapidly than did the phosphorous. Urinary excretion of calcium was increased 60 minutes after injection. Phosphorous excretion had two maxima, one at 60 minutes and the other 5 hours following injection. Localization of P^{32} in these animals was primarily in the liver and kidney with smaller amounts in lung and spleen. Five minutes after the injection of labeled colloidal chromium phosphate the specific activity of the blood was negligible. No significant changes in serum calcium were observed. The urinary excretion of calcium decreased slightly during the 30 minutes following injection while the phosphorous excretion increased sharply between the 4th and 6th hour. The P^{32} was found to be concentrated in the liver, lungs and spleen with no localization in the kidney. From these observations it seems that the removal of calcium phosphate and chromium phosphate may be governed by different mechanisms.

Vitamin B_{12} activity of 5,6-dimethylbenzimidazole, 1,2-diamino-4,5-dimethylbenzene and riboflavin for the rat. J. M. COOPERMAN AND B. TABENKIN (introduced by S. H. RUBIN). *Depts. of Nutrition and Microbiology, Hoffman-La Roche, Inc., Nutley, N. J.*

Rats maintained on the vitamin B_{12} depletion diets of Register *et al.* (*J. Biol. Chem.* 177: 129, 1949) and modified so that 100 mg. crystalline thyroxine per kg. replaced the iodinated casein, and of Emerson (*Proc. Soc. Exper. Biol. & Med.* 70: 392, 1949) were given 5,6-dimethylbenzimidazole (*A*), 1,2-diamino-4,5-dimethylbenzene (*B*) and riboflavin (*C*) to determine the vitamin B_{12}-like activity of these compounds. When administered interperitoneally *compound B* had a very low order of activity. Under these conditions *compounds A* and *C* can partially satisfy the vitamin B_{12} requirement. Ten or 15 rats were used in each group. These results are shown in the following table.

SUPPLEMENT PER DAY	TOTAL GAIN 2 WEEK ASSAY PERIOD—GM.	
	Diet of Register et al.	Diet of Emerson
0	34	24
0.05γB_{12}	55	53
0.20γB_{12}	77	71
1 mg. *A*	46	34
5 mg. *A*	46 (1 dead)	
2 mg. *B*	34	30
3 mg. *B*	41	
2 mg. *C*	50	

Preliminary experiments that *compound A* may be somewhat more effective when given orally, but no increase in effectiveness was noted for *compound B* when it was administered orally.

Antagonism of anethole and adrenal extract on glycogen formation in adrenalectomized rats. LUCILLE COTÉ AND J. J. OLESON. *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

A study was made of the effect of various substances on the deposition of glycogen in the livers of bilaterally adrenalectomized, fasted rats by lipoadrenal extract. Anise oil and its major constituent, anethole, were found to strongly inhibit glycogen formation. This inhibition was reversible. For example 100 mg. of anethole inhibits the effect of 200 γ of lipoadrenal extract and is reversed by 1 mg. of the extract. Other active compounds include menthol, borneol, anisic acid, citral, vanillin and eugenol. It is known that these compounds are detoxified by conjugation to glu-

curonides. Thus, they may act in part by competing for available glucose. If 100 mg. of exogenous glucose is given with the adrenal extract, glycogen deposition is increased. This effect is also blocked by 200 mg. of anethole. The effect of adrenal extract in decreasing insulin sensitivity is also blocked by anethole. The latter has little effect alone on the production of hypoglycemia by insulin. Anethole increases adrenal hypertrophy in unilaterally adrenalectomized animals. Thus it may increase bodily demand for adrenal steroids.

Egg white proteins. FRANK A. CSONKA AND MERRIAM A. JONES.* *Bureau of Human Nutrition and Home Economics and Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA, Washington, D. C.*

Electrophoretic studies were conducted to determine the influence of diet and of the genetic lineage of the hen on the protein composition of egg white. Two dietary regimens differing in their protein sources were studied. Determinations were made of the ovomucoid, ovalbumen, conalbumen and globulin content of eggs laid by pullets of Rhode Island Red-Leghorn crosses and of standardized bred Rhode Island Reds. Differences were observed in the composition of the egg white of the eggs from the two genetic lines of hens studied. The major differences observed were in the ovomucoid, conalbumen and globulin content of the eggs and were noted whether the hens were on a diet of relatively low or of relatively high protein content.

In vitro conjugation of phenol by rat liver. R. H. DEMEIO (introduced by W. H. PEARLMAN). *Dept. of Biochemistry, Jefferson Med. College, Philadelphia, Pa.*

Rat liver slices produce phenylglucuronide and phenylsulfate, whereas homogenates fortified with adenylic and alpha-ketoglutaric acids conjugate most of the phenol with sulfate; more phenylglucuronide is produced by the slices in absence of sulfate. Phenyl sulfate formation increases more rapidly with time of incubation than phenylglucuronide. Maximum conjugation with slices is attained in 4 hours and in one hour with homogenate. Phenylsulfate is not hydrolized to an appreciable extent by slices or homogenates in presence or absence of cyanide or sulfate. Other phenols inhibit conjugation by slices in decreasing order of activity: dinitrophenol, *o*-nitrophenol and salicylate, acetylsalicylate, *l*-tyrosine. In a sulfate-free medium conjugation by slices is not affected by the addition of taurine and glutathione, but is considerably increased by that of cysteine and thiosulfate. The homogenate is active in oxygen but not in air. The same activity is observed when sodium predominates as when potassium is the main cation in the medium and when alpha-ketoglutarate is replaced by succinate. A sediment and a supernate are obtained by centrifugation of fortified homogenate at 600 *g*. If separately resuspended in fortified medium, no conjugation occurs, but it does when they are recombined (but not if the supernate is previously boiled.)

Reactions of xanthydrol with certain amino acids and proteins. SHERMAN R. DICKMAN AND WAYNE L. WESTCOTT (introduced by LEO T. SAMUELS). *Dept. of Biological Chemistry, Univ. of Utah College of Medicine, Salt Lake City.*

Xanthydrol in acetic acid solution forms derivatives with asparagine, glutamine, arginine and lysine. Alanine, leucine, threonine and tyrosine did not react. The ultraviolet spectral-absorbancy curves of the xanthyl amino acids have a broad band in the range 2700–2900 Å and also exhibit high absorbancies below 2500 Å. They thus resemble the spectral-absorbancy curve of xanthydrol itself which shows a peak at 2890 Å and maximum absorbancy below 2500 Å. The product formed by the treatment of cytochrome *c* with xanthydrol in acetic acid at room temperature for 30 minutes was catalytically inactive when tested in the cytochrome oxidase-succinic dehydrogenase system. When xanthyl-cytochrome *c* was assayed by the chemical spectrophotometric method, however, practically no inhibition was found. Dickman and Proctor (unpublished) previously demonstrated that xanthydrol inhibited egg white lysozyme. Xanthydrol also reacts with insulin in acetic acid. The ultraviolet spectral-absorbancy curve of the aqueous phase after ether extraction had a much higher absorbancy at 2750–2900 Å than the original insulin solution.

Inhibition of anaerobic and aerobic phosphorylation of glucose in nucleated red cells by intracellular ions. ZACHARIAS DISCHE AND GILBERT ASHWELL.* *Dept. of Biochemistry, Columbia Univ. College of Physicians and Surgeons, New York City.*

The mechanism of the inhibitory effect, previously described, of intracellular ions on the aerobic glycolysis in hemolysates of pigeon erythrocytes was further investigated. The anaerobic glycolysis of glucose in hemolysates freed of organized particles by high-speed centrifugation goes on only in presence of ATP. It is inhibited by multivalent anions (Na_2SO_4 M/50, KxFe/CN/6 M/250, Na Phosphate M/50, Na Citrate M/100, ribonucleate 0.5%). Ca^{++}, Ba^{++} M/200 La^{+++} M/500 NaCl and K Cl M/10 do not inhibit or accelerate. Hexokinase and two other enzymes of the glycolytic system were shown to be the points of attack of

the inhibitors. The aerobic glycolysis in non-centrifuged hemolysates is inhibited by lower concentrations of multivalent anions than are effective against the aerobic glycolysis, and by multivalent inorganic cations at concentrations ranging from M/5000 to M/1000. The respiration is also inhibited by the multivalent ions, but to a lesser degree than the aerobic glycolysis. As the aerobic breakdown of glucose was shown to be preceded by its phosphorylation our experimental data indicate, that multivalent ions inhibit the aerobic phosphate transfer to a phosphate carrier, which phosphorylates glucose. This inhibition appears at concentrations of multivalent ions, which can be considered as physiological. Anions and cations show a pronounced synergy in their inhibitory effect on the respiration as well as the aerobic glycolysis. (This work has been supported by a research grant from the Donner Foundation.)

An improved method for preparing sodium desoxypentose nucleate. ALEXANDER L. DOUNCE, NORMAN S. SIMMONS* AND ERNEST R. M. KAY.* *Depts. of Pathology and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

A convenient procedure has been developed for isolating sodium desoxypentose nucleate (Na-D.N.A.) using sodium dodecyl sulfonate (S.D.S.) which extracts firmly bound Na-D.N.A. from animal tissue and which leads to very complete removal of protein from the product. Animal tissue (or isolated cell nuclei) is extracted repeatedly with 0.9% NaCl in the presence of 0.01 M citrate. The residue is extracted for 3 hours with 0.45% S.D.S. in 0.9% NaCl. The NaCl concentration is brought to 5% and the suspension is clarified by centrifugation. Na-D.N.A. is precipitated from the supernatant by adding 1 volume of alcohol. The crude product is dissolved in water and stirred for 1 hour after adding S.D.S. (0.45% in concentration). NaCl is added until 5% in concentration, the suspension is clarified by centrifugation at high speed and the Na-D.N.A. is precipitated by adding one volume of alcohol. The precipitated product is dissolved again in water, NaCl is added until 0.9% in concentration, and the solution is centrifuged at high speed. Finally, the Na-D.N.A. is precipitated from the supernatant by adding more NaCl and alcohol as previously. The product gives negative tests for protein and is satisfactory from an analytical standpoint. The degree of polymerization is very high as evidenced by the formation of a fairly stiff gel in distilled water at a concentration of only 0.3% Na-D.N.A.

Production of red cells in acclimatization to altitude—comparative study of in vitro systems in biosynthesis of hemoglobin. DAVID L. DRABKIN AND ASHTON GRAYBIEL. *Dept. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, and U. S. Naval School of Aviation Medicine, Pensacola, Fla.*

Decisive information is unavailable as to whether, in acclimatization to oxygen lack at high altitude, pre-fabricated erythrocytes are extruded into the circulation from tissue stores or whether new red cells are produced, either in the bone-marrow or some extra-medullary locality. Our studies were upon similar thoroughbred Beagle hounds on a standard diet and were carried out over extended periods of time to furnish clear-cut definition of features in the acclimatization phenomenon. Identical changes in erythrocyte, hemoglobin and hematocrit values were found in a splenectomized animal and its control observed for 1500 hours at a simulated altitude of 15,000 feet and 1000 hours of readjustment at sea level, except in the first hour at high altitude, during which time only may an inference of splenic involvement be made. The following significant activities (in counts/minute/mg.) were obtained upon samples of thrice recrystallized hemin prepared from blood incubated *in vitro* with glycine-2-C^{14}: duck blood, 37.8, blood from dogs acclimatized to 15,000 feet, 7.67, 12.43, and blood from a control dog at sea level, 4.45. The standard error of the net counts was ±2%, and 'quasi incorporation' was ruled out. The evidence suggests that the production of new cells is a major component in the characteristic acclimatization response, and, furthermore, that the hemoglobin biosynthetic capability of blood *in vitro* is a relative matter, and not confined to avian erythrocytes.

Effect of 2,6-diaminopurine on purine metabolism in *Neurospora*. WILLIAM DRELL (introduced by N. H. HOROWITZ). *Kerckhoff Labs. of Biology, California Inst. of Technology, Pasadena.*

The effects of diaminopurine, guanine, guanosine, isoguanine and isoguanine riboside on the growth of adenineless *Neurospora* including strains 27663 and 28610 have been studied in the presence of suboptimal amounts of adenine, adenosine, hypoxanthine or inosine. Diaminopurine, at relatively low concentrations, completely inhibits the growth of either mutant in the presence of adenine or hypoxanthine but not in the presence of adenosine or inosine. It is approximately tenfold more inhibitory when hypoxanthine rather than adenine is used. The inhibition is reversed by either purine. Guanine inhibits growth entirely in the presence of hypoxanthine while guanosine

prevents the utilization of inosine as well. Under certain conditions diaminopurine, guanine and guanosine exert a sparing action on the utilization of adenine or adenosine. Isoguanine and its riboside are relatively inactive in all cases. The role of diaminopurine riboside is under investigation. The relation of these findings to the biosynthesis of purines in *Neurospora* will be discussed.

Vitamin B_{12} in methionine formation. JACOB DUBNOFF. *California Inst. of Technology, Pasadena.*

It was previously reported (*Federation Proc.* 9: 166, 1950 and *Arch. Biochem.* 27: 466, 1950) that B_{12} concentrates catalyze the reduction by rat liver slices of homocystine to homocysteine. Further work has shown that crystalline B_{12} in concentrations as low as 0.0001 γ/ml. is also active. Reduced glutathione markedly increases the reduction of homocystine. Independent evidence confirming this role for B_{12} in methionine synthesis based on the replaceability of B_{12} in the B_{12} or methionine requiring mutant of B. Davis by homocysteine will be reported.

Radioautographic visualization of sulfur-35 disposition in articular cartilage and bone of suckling rats following administration of labeled sodium sulfate. DOMINIC D. DZIEWIATKOWSKI. *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

The osseous localization of sulfur-35 administered intraperitoneally as Na_2SO_4 to 7-day old rats was determined by radioautography of sections of humeri removed at 24, 48, 96, 216, and 290 hours after injection of the isotope. After 24 hours, in the tissue that had been fixed in formalin, the isotope was found distributed throughout the entire epiphysis. Its concentration therein was highest initially at the epiphyseal-diaphyseal junction. By the 96th hour there were indications that an equalization of the isotope concentration in the cartilage was in progress. An equal distribution of the sulfur-35 throughout the epiphyseal cartilage was almost attained by the 216th and 290th hour. As centers of secondary ossification arose in the epiphyses, the sulfur-35 appeared to diminish in concentration and disappear from these loci. Radioautographs of cartilage fixed in formalin saturated with $Ba(OH)_2$, instead of only in formalin, disclosed the fact, however, that the isotope was still present in the centers of secondary ossification. When bone and bone marrow were fixed in formalin the autographs indicated the presence of the sulfur-35 primarily in the periosteum with only a negligible amount in the bond shaft and marrow. However, when these tissues were fixed in formalin saturated with $Ba(OH)_2$ it was possible to demonstrate that the isotope was definitely present in the bone and bone marrow.

Microbiological examination of mixtures of amino acid derivatives and amino acids. CHARLES H. EADES, JR. (introduced by T. P. NASH, JR.). *Div. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis.*

Most microbiological assay techniques do not differentiate the free amino acids from the 'combined' utilizable forms of amino acids. Data are presented showing that certain derivatives of amino acids may be detected (microbiologically) in the presence of the free amino acids. The procedures involve the use of several commonly employed analytical microorganisms in parallel assay. The differing specificities in amino acid requirements and utilization of derivatives are made the bases for the determinations. Application of these techniques to the study of biological fluids is discussed.

Effect of negative ionization on cellular proliferation in connection with calcium retention. WALTER H. EDDY AND BORIS SOKOLOFF.* *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

It is known that the calcium content of most malignant tissue is decreased. In benzpyrene induced cancer there is a definite diminution of calcium in the transformed cells and tissues. It was suggested that the change in pH might be responsible for this modification in calcium content. Negative ionization induces electrolytic changes and affects the pH of tissue. In the present work the effect of negative ionization on transplanted rat carcinoma 175-G and mouse adenocarcinoma Eo-771 was investigated. The animals were placed in a special cubicle the day of transplantation. The density of negative ions per cc. of air was maintained at about 6000–8000 level. The animals were kept in these cubicles continuously, except for short intervals required for feeding, for 20–25 days. Considerable inhibition was observed in rat carcinoma 175-G. At the end of 3 weeks the average size of tumor of so-treated animals was 5.2 cm.^{3} as against 9.9 cm.^{3} of control. At the end of 4 weeks, the average size was 6.5 cm.^{3} as against 12.5 cm.^{3} A greater inhibition was achieved when the rats kept in the atmosphere of negative ionization were given calcium lactate, 5 mg/100 gm. wt. daily. While calcium lactate produced only a slight inhibition (average size 9.9 cm.^{3} as against 10.4 cm.^{3} at the end of 4 weeks after transplantation) the average size of tumors of the rats kept under negative ionization and given calcium had remained almost stationary: 2.7 cm.^{3} at the end of 4 weeks after the transplantation. An investigation to determine if nega-

tive ionization enhances the retention of calcium in malignant tissue is under way.

Studies in the series of adrenal cortical hormones: dehydration of 6β, 21-diacetoxyallopregnane-5-ol-3,20-dione. MAXIMILIAN EHRENSTEIN AND PABLO TH. HERZIG.* *Dept. of Research Medicine and George S. Cox Med. Research Inst., Univ. of Pennsylvania, Philadelphia.*

As the final step in a series of reactions, Ehrenstein (*J. Org. Chem.*, 6: 626, 1941; 13: 214, 1948) reported the dehydration of 6β, 21-diacetoxyallopregnane-5-ol-3,20-dione (I) with dry hydrogen chloride in chloroform. The resulting product (m.p. 84–88°; [α]D + 114.3°) was interpreted to be 6β-hydroxy-11-desoxycorticosterone 6,21-diacetate (II). Recent evidence indicates that the latter product actually is 6α-hydroxy-11-desoxycorticosterone 6,21-diacetate (III). A reinvestigation showed that on treating I with dry hydrogen chloride in ordinary chloroform (i.e., containing ethanol), a mixture of two isomers, II (m.p.125–127°; [α]D + 98°) and III (m.p.84–86°; [α]D + 120°), results in which frequently III predominates. Treatment of I with Girard's Reagent T or with hydrogen chloride either in carbon tetrachloride or alcohol-free chloroform yields exclusively II which rearranges to III by the action of hydrogen chloride in ordinary chloroform or in carbon tetrachloride containing 0.7% ethanol. Treatment of II and III with absol. ethanolic potassium hydroxide in an atmosphere of nitrogen yielded 6β-hydroxy-11-desoxycorticosterone (IIa) and 6α-hydroxy-11-desoxycorticosterone, (IIIa) respectively. On the other hand, by the action of absolute ethanolic potassium hydroxide in an atmosphere of oxygen exactly one mole of oxygen is consumed (cf. VELLUZ *et al. Bull. Soc. Chim. France* 123, 1947) resulting in the practically quantitative formation of 3-keto-6β-hydroxy-Δ^4-etiocholenic acid (IV) and 3-keto-6α-hydroxy-Δ^4-etiocholenic acid (V), respectively. The latter can also be obtained by the action of periodic acid on IIa and IIIa, respectively. Treatment of II with aqueous potassium hydroxide results in saponification and rearrangement leading to allopregnane-21-ol-3,6,20-trione (VI) which in turn can be transformed into 3,6-diketoetioallocholanic acid (VII). Several additional reactions will be discussed.

Precipitin reaction between hapten-azo-protein and anti-hapten rabbit sera. HERMAN N. EISEN (introduced by NORTON NELSON). *Inst. of Industrial Medicine, New York Univ.-Bellevue Med. Center, New York City.*

Quantitative study has been carried out of the precipitin reaction involving 2,4 dinitro-phenyl-(D-) azo-ovalbumin (Ea) and rabbit antisera prepared against D-azo-beef serum proteins. D-azo-proteins were prepared by coupling D-diazonium salt in 50-fold excess with protein in acetate buffer, pH 5.0. Removal of uncoupled reagent was achieved principally by charcoal extraction, using an acetone spot test to follow purification. Homogeneity was indicated by optical density-nitrogen ratios (O.D./N) after successive extractions, and after dialyses. From the molar extinction coefficient of D-azo-*p*-cresol and O.D./N of D-azo-Ea, the mol ratio of D-azo to Ea was estimated. Maximally, this ratio was 12. Precipitates formed by D-azo-Ea and anti-D-azo sera were analyzed spectrophotometrically and by Kjeldahl. Recovery of precipitate was influenced by precipitation time and by temperature of washing in agreement with results of Heidelberger *et al.* on protein-antiprotein and polysaccharide-antipolysaccharide systems. This hapten-antihapten system differs from the foregoing systems in three respects: *a*) precipitation of D-azo-Ea is never complete in antibody excess zone; *b*) dilution of antigen-antiserum mixture decreases amount of precipitate formed; *c*) antibody-antigen composition (B/G) of precipitates varies systematically as a *non*-linear function of non-precipitated antigen over a broad range (B/G varying 5–10 fold). Comparison of sera after successive immunization courses indicated changes in antigen combining properties; successive sera from some rabbits had progressively decreasing concentration of precipitating antibody, and eventually no precipitating antibody, even after only 2–4 months of immunization.

Degradation of biosynthetic glucuronic acid labeled with C^{14}. FRANK EISENBERG, JR. AND SAMUEL GURIN. *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

The application of the isotopic tracer technique to the study of the biosynthesis of glucuronic acid has led to the need for a chemical degradation method designed to locate the position of the isotope in the chain. Thus far only the classical decarboxylation yielding C_6 has been used (MOSBACH AND KING, *J. Biol. Chem.* 185: 491, 1950). Two new procedures have been developed producing five additional fragments; $C_{1.6}$, $C_{2.5}$, $C_{3.4}$, $C_{1.2}$, and $C_{4.5.6}$. Oxidative hydrolysis of menthyl glucuronide with Br_2 and HBr yields saccharic acid, which when treated with HIO_4 breaks into 2 moles of formic acid from $C_{3.4}$. Glyoxylic acid was further degraded by known procedures to CO_2 and HCOOH representing, respectively, $C_{1.6}$ and $C_{2.5}$. Methyl menthyl glucuronidate on oxidation with HIO_4 yields L'-(L-menthoxy)-D-carbomethoxy-diglycolic aldehyde. Hydrolysis of the product with semicarbazide hydrochloride yields

the semicarbazones of glyoxal, representing $C_{1,2}$, and mesoxalaldehyde methyl ester, representing $C_{4,5,6}$. These degradations, as well as decarboxylation, have been applied to radioactive menthyl glucuronide isolated from rabbit urine after separately feeding $NaHC^{14}O_3$, $C^{14}H_3C^{14}HOHCOOH$, and $CH_3CHOHC^{14}OOH$. Preliminary results indicate symmetrical incorporation of C^{14} into $C_{3,4}$, $C_{1,2,5,6}$, and $C_{3,4}$, respectively.

Effect of addiction to morphine on excretion of water and electrolytes. ANNA J. EISENMAN. *Research Branch, US Public Health Service Hosp., Lexington, Ky.*

Two subjects were placed on a low sodium diet. Three control days were followed by 20 days when they received 30 mg. of morphine 4 times daily. The subjects drank 1500 cc. of water and had an intravenous injection of 1000 cc. of glucose daily. On the first day of addiction there was a 60–90% decrease in the excretion of water, sodium and chloride. *Subject 1* showed decreased excretion for 4 days, reached control values on the 5th day, exceeded them on the 6th day. Thereafter, excretion of these substances was practically normal, with minor day to day variations. In *Subject 2*, water excretion was decreased for the first day only. For 6 days, there was very low sodium and chloride excretion. Thereafter, from the 8th to the 20th day, he showed a fluctuating excretion of salt, in general higher than during the control period. During sodium retention, potassium excretion exceeds its normal ratio of about 40% of the sodium excretion. In one subject it reached 500% at one time and did not regain the normal ratio until the 7th day. In the other subject, it had returned to its normal ratio in about 3 days. This is probably not an addiction effect, since the same phenomenon has been observed in other conditions of sodium and water retention. In general, it may be said that tolerance to the antidiuretic effects (retention of water, sodium and chloride) of morphine is reached after about one week of addiction.

Effects of chemical modifications on antimetabolite activities of 2,6-diaminopurine. GERTRUDE B. ELION (introduced by G. H. HITCHINGS). *Wellcome Research Labs., Tuckahoe, N. Y.*

The strong inhibitory effect of 2,6-diaminopurine on the growth of *Lactobacillus casei* and the behavior of diaminopurine as a competitive antagonist of adenine has led to the study of 30 derivatives of 2,6-diaminopurine, substituted in various positions on the imidazole ring as well as on the amino nitrogens. All of these compounds, with one or two exceptions, are inhibitors of the growth of *Lactobacillus casei* in a folic acid-containing medium. These effects are reservible partially or completely by either folic acid or adenine. The pattern of these reversals is, however, different from that of 2,6-diaminopurine itself and is dependent on the nature and position of the substituent. For most of these compounds the inhibitions are overcome equally well by adenine, guanine, xanthine and hypoxanthine; for a few of the compounds there is some degree of specificity in the purine reversals but in no case is this specificity comparable to that shown by adenine in overcoming the action of 2,6-diaminopurine.

Serum sodium and potassium values in 400 normal human subjects determined by Beckman flame photometer. HOWARD C. ELLIOTT, JR. AND HOWARD L. HOLLEY (introduced by EMMETT B. CARMICHAEL. *Biochemistry Dept. and Dept. of Medicine, Alabama Med. Center, Birmingham.*

There has been an obvious need for a study of a large series of human subjects to establish a 'normal' range and mean. This report is concerned with a statistical study of the results on 400 sera obtained from fasting volunteers at the Jefferson-Hillman Hospital blood bank, Birmingham. Samples were examined by the technique described by Mosher *et.al.* (*Am. J. Clin. Path.* 19: 461, 1949) with slight modifications. Duplicate determinations were made, and the average of two readings on each determination was taken as the correct reading. Experimental error was studied and results indicate that the average deviation percentage of average is 0.86% for the sodium and 0.79% for the potassium determinations. Sodium values range from 135.0–155.0 mEq/l, with an average of 144.7. Potassium values range from 3.1–5.5 mEq/l. with an average value of 4.18 mEq/l. The ranges covered by one standard deviation in both sodium and potassium determinations are in accordance with Arkin and Colton (*Statistical Methods*, 4th Ed., 1939) who state that 68.26% of the values should be covered by the mean ± one standard deviation in a normal distribution curve. The sodium values are in excellent agreement with those previously reported by other workers. (SMITH *et. al. Am. J. Clin. Path.* 20: 263, 1950; MARINIS *et. al. J. Lab. & Clin. Med.* 32: 1208, 1948; OVERMAN *et al. J. Biol. Chem.* 168: 641, 1947; HALD, *J. Biol. Chem.* 167: 499, 1947). The average value for potassium falls significantly below that reported by most workers but is in agreement with the results reported by Smith.

Nucleotide composition of pentose nucleic acids from different fractions of the hepatic cell. DAVID ELSON* AND ERWIN CHARGAFF. *Dept. of Biochemistry, Columbia Univ., New York City.*

An adaptation of methods for the separation and micro estimation of ribonucleotides previ-

ously developed in this laboratory, has been applied to the direct study of the ribonucleic composition of cellular elements without isolation of the nucleic acid. Studies thus were carried out on the distribution of nucleotides, present in readily extractable form and as polymerized nucleic acids, in nuclei, mitochondria, submicroscopic particles of different sizes and other fractions of rat liver. The pentose nucleic acids of the cytoplasmic fractions appear to be similar to liver nucleic acid preparations analyzed previously (CHARGAFF *et al. J. Biol. Chem.* 186: 51, 1950) and are characterized by a high content of guanylic and cytidylic acids. The pentose nucleic acid of the nucleus, presumably derived from the nucleolus, is differently constituted. In two preparations the following results were obtained (with adenylic acid as 10): guanylic acid 11.0, 11.3; cytidylic acid 15.4, 15.7; uridylic acid 10.9, 10.3. Similar methods were, in collaboration with Dr. T. Gustafson of Stockholm University, applied to the study of nucleic acid composition in sea urchin eggs in different stages of embryonic development.

Utilization of host phosphorus in virus reproduction. E. A. EVANS, JR., FRANK W. PUTNAM AND DEAN MILLER.* *Dept. of Biochemistry,* Univ. of Chicago, Chicago, Ill.*

Bacteriophage T_7 was grown by multiple infection of *E. coli* in lactate medium. Phage were adsorbed on P^{32}-labeled cells in Ringer's solution and the washed infected bacteria suspended in unlabeled medium, or conversely, unlabeled bacteria were similarly infected and put into radioactive medium. Titers and light transmission remained constant for 50–60 minutes, then increased sharply with a concomitant maximum in ultraviolet light scattering followed by rapid lysis of the bacteria. The specific activity of the bacterial P fractions—acid soluble, alcohol soluble, ribonucleic acid (RNA), and desoxyribonucleic acid (DNA)—was followed kinetically and the activity of the purified ultracentrifugally homogeneous bacteriophage was also determined. An immediate change in activity of acid soluble P was noted (presumably due to exchange) which led to differentially labeled cells in all experiments. With labeled bacteria the specific activity of purified phage was twice that of bacterial acid soluble P and 75% that of other P fractions. With bacteria differentially labeled by growth in two media, virus specific activity was 5 times that of host acid soluble P and 85–100% that of other P components. With labeled medium and differential incorporation of P^{32} the specific activity of the virus was 18% that of the medium, 36% that of host acid soluble P, 69% that of RNA, and 100% that of DNA. No net increase in DNA was observed, and total phage DNA was equal to 20–50% of initial bacterial DNA. The results show, in agreement with experience with T_6 bacteriophage, that virus DNA arises from bacterial DNA, not from the bacterial acid soluble P present at infection. In contrast to T_6, most or all of the P of T_7 bacteriophage is derived from the host.

Relation of tryptophan and niacin to liver pyridine nucleotides. PHILIP FEIGELSON AND J. N. WILLIAMS, JR. (introduced by C. A. ELVEHJEM). *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Recent studies in our laboratory have indicated that rats fed tryptophan-free diets developed, in spite of high dietary niacin levels, an abnormally low concentration of liver pyridine nucleotides (PN). Dietary supplementation with tryptophan restored the liver PN level to normal, whereas niacin supplements exerted only slight effects. To check the possibility that tryptophan deficiency decreased the synthesis of enzymes responsible for incorporation of niacin into PN we have studied the effects on liver PN of tryptophan and niacin supplements to rations which are otherwise completely protein-free. Under these conditions a direct comparison of niacin and tryptophan as precursors of liver PN could be studied. Weanling and adult rats were placed on a non-protein ration until their liver PN were depleted. Tryptophan and niacin supplements then were added and fed for two weeks after which the animals were sacrificed and the liver PN measured. The adult rats did not respond to the niacin supplements. Their liver PN values did not increase above the group getting no supplements, whereas with tryptophan supplements the liver PN level rose considerably. The weanling rats did respond somewhat to extremely high supplements of niacin though equimolar amounts of tryptophan resulted in still higher liver PN values. It therefore appears that tryptophan does not stimulate PN synthesis due to its incorporation into enzyme proteins, but more likely serves as a PN precursor, of apparently greater importance than niacin.

Mechanism of holophosphorylase action. IRWIN FEIGIN, JEROME FREDRICK AND ABNER WOLF (introduced by HANS T. CLARKE). *Veterans Administration Hosp., Bronx, Depts. of Pathology and Neurology, Columbia Univ., and Neurological Inst., New York City.*

Crystalline holophosphorylase (A), a glycoprotein, is believed to act by incorporating glucose from glucose-1-phosphate into its own molecule, liberating inorganic phosphate. The product (B) precipitates when ethanol is added to the incubation mixture to a concentration of approximately 20% by volume. A, which precipitates at ap-

proximately 30% ethanol, is not precipitated in the filtrate remaining after the removal of B, although mixtures of A and B can be separated by fractional precipitation with alcohol. B resembles glycogen in giving a red-brown color with iodine, in being soluble in water, in failing to reduce copper reagents until after acid hydrolysis, and in 'priming' the action of muscle phosphorylase. After treatment with alkali, B, like glycogen, yields no precipitate with alcohol until a concentration of 50% is reached. B, incubated aerobically with inorganic phosphate, yields glucose-1-phosphate. With low substrate concentrations, A yields intermediate substances which are also glycoproteins, containing more carbohydrate and less nitrogen than A, and are precipitated by lower concentrations of alcohol. With high substrate concentrations, variation in concentration of A results in variation in quantity of B, as well as in rate of reaction. B may be a form in which glycogen is naturally present in muscle. B and A appear to differ only in the proportion of carbohydrate in the molecule. Glycogen formation and glycogenolysis may consist of the addition and loss, respectively, of glucose residues to and from this molecule.

Action of thyroxine in tissue oxidations. GLADYS FELDOTT* AND HENRY A. LARDY. *Dept. of Biochemistry and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

A study has been made of the *in vitro* effect of thyroxine on the oxidation of glutamic acid, Krebs' cycle intermediates, DL-β-hydroxybutyric acid, and various fatty acids by homogenized rat tissue preparations. Thyroxine at a concentration of 10^{-5}M, in most cases, inhibited the O_2 uptake by a washed residue of rat kidney cortex in the presence of glutamate and Krebs' cycle intermediates. Inhibition of glutamic acid oxidation was most marked, often apparent at 10^{-6}M thyroxine, and therefore a more detailed study was made with this substrate. Cytochrome *c*, ATP, and CoI effected a partial, but never complete, reversal of the thyroxine inhibition. Analogs of thyroxine which are biologically active (FRIEDEN AND WINZLER. *J. Biol. Chem.* 176: 155, 1948) also exerted an inhibition similar to that of thyroxine on the glutamate oxidation system. Fractionation of the enzyme preparation indicated that the response to thyroxine was more pronounced in the nuclear fraction. Only with extremely low enzyme concentrations was the effect observed in the mitochondrial fraction. Thyroxine stimulated the oxidation of DL-β-hydroxybutyric acid and this increased rate of O_2 uptake was accompanied by an increased rate of acetoacetate disappearance. With washed residues from rat kidney, the effect of thyroxine on fatty acid oxidation was variable; tremendous stimulation of activity occurred in some experiments.

Effect of calcium on rigidity and stress relaxation of fibrin clots. JOHN D. FERRY, MEREDITH MILLER* AND SIDNEY SHULMAN.* *Dept. of Chemistry, Univ. of Wisconsin, Madison.*

The dynamic rigidities of bovine fibrin clots have been measured by the method of transverse wave propagation, and the relaxation of stress and reverse stress relaxation have been followed in a concentric cylinder apparatus by procedures recently developed in the study of gelatin gels (*J. Phys. & Colloid Chem.*, in press). In the absence of calcium, at pH 7, the wave rigidity is proportional to the 2.4 power of fibrin concentration; and it decreases reversibly with increasing temperature over the range from 5° to 30°C., suggesting a reversible dissociation of bonds. After a small sudden strain the stress relaxes rather rapidly, also indicating bond dissociation or rearrangement; in a 1% clot at 10°C. it decays 80% in 10 minutes. That some of the broken linkages reform is evidenced by a reverse stress which appears when the strain is returned to zero and then in turn relaxes. The relaxation behavior at pH 6.2 is similar. In the presence of 10^{-3} M calcium, however, the stress relaxes much more slowly, at 10° decaying only 20% in 250 minutes. With calcium, the rigidity is much less temperature-dependent. Thus fibrin formed in the presence of calcium appears to lack the reversibly dissociable linkages. In agreement with Robbins and with Lorand, only the calcium-free clots are soluble in concentrated urea and in dilute acid; it is inferred that our fibrinogen preparations contained the serum factor described by these investigators.

Effect of choline deficiency on serum proteins of the young rat. MARIE A. FISCHER (introduced by W. S. MCELLROY). *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pittsburgh, Pa.*

To produce an acute choline deficiency, groups of female, weanling rats of the Sherman strain were placed on a purified diet containing 18% casein, 20% Crisco and 0.3% l-cystine for periods varying from 0 to 25 days. Two series of control groups were also fed the same diet, but, in addition, received either a 2 mg. or a 6 mg. daily oral supplement of choline hydrochloride. The animals in each group were sacrificed by decapitation and their blood was pooled by collection into a single beaker. Serum was prepared from the pooled blood and the protein content was determined by electrophoresis. Compared with a series of animals maintained on Purina Chow, the albumin areas of the experimental groups showed greater fluctua-

tion. However, the three series of animals on the purified ration all showed an initial decrease in serum albumin followed by a rise on the 4th day and a subsequent decrease to a low level by the 6th day. The rise from this low level was abrupt in those animals receiving the supplements, but was gradual in the unsupplemented groups, 90% of which had developed kidney lesions between the 5th and 7th days. Following the development of the kidney lesions, a 2-fold increase in both the α_1 and α_2-globulin areas was evident. A lesser increase also occurred in the β_1-globulin area. This rise in the serum globulin fractions is probably a result of the kidney damage and after kidney recovery, the globulin levels return to normal.

Free amino acids of plasma following complete removal of the liver. EUNICE V. FLOCK, FRANK C. MANN* AND JESSE L. BOLLMAN.* *Div. of Exptl. Medicine, Mayo Fndn. Rochester, Minn.*

Following complete removal of the liver there is a gradual accumulation of free amino acids in the blood, tissues and urine. There is no urea formation and amino acids are not converted to glucose nor do they show specific dynamic action. The accumulation of amino acid nitrogen is less than half the equivalent of the amount of urea nitrogen formed over the same period of time by control dogs. Two-dimensional chromatograms made from concentrated desalted dialysates of plasma and urine removed from dogs at intervals up to 31 hours after complete removal of the liver indicated the following conclusions. The relative distribution of individual amino acids in the blood after liver removal is not greatly altered and the increase is shared by both essential and non-essential amino acids. No amino acid or its derivative appeared to increase predominantly or at the expense of other amino acids. The blocking of urea formation after liver removal seems to be at the amino acid stage. The rate of accumulation of amino acids in the plasma after hepatectomy varies somewhat and appears to be influenced by some factors which also influence the concentration of amino acids in the intact dog. Conjugation of amino acids occurs after liver removal and a large portion of the amino acids excreted in the urine are in the conjugated form.

A trypsin-resistant lipide-protein complex isolated from brain white matter proteins. J. FOLCH AND F. N. LE BARON.* *McLean Hosp. Waverley, Mass. and Harvard Med. School, Boston, Mass.*

After digestion of white matter proteins (previously freed of lipides, proteolipides, and acid-soluble components by presumably adequate treatment) with trypsin, a trypsin-resistant residue is obtained (Yield: 4 gm/kg. of starting tissue). It contains 11.0 % N, 1.60 % S, and 1.77 % P. Its N represents protein material. Its P is present as inositol diphosphate which itself is in combined form, probably as a constituent of a phosphatide closely related to, or identical with diphosphoinositide. In spite of its lipide nature, this P cannot be extracted by treatment of the material with solvents short of prolonged extraction with ethanol in a Soxhlet. This type of extraction removes P slowly (70 % of the P removed in 7 weeks), and it exerts a chemical action on the material, one-fourth of the P extracted having become inorganic P. Thus it appears that a lipoprotein complex is being dealt with in which the bond between the constituent lipide and protein moieties is much stronger than is usually the case in complexes of this type. The material is resistant to the action of trypsin, papain, and erepsin. Its P accounts for 40 % of the P that is left in white matter after removal of lipide P and acid-soluble P by procedures commonly used. Under the conditions employed in the method of Schmidt and Thannhauser (J. Biol. Chem., 161: 83, 1945) for nucleic acids estimation, this P appears quantitatively in the ribose nucleic acid P fraction.

Vitamin B_T. I. Deficiency symptoms, method of testing, distribution, isolation procedures and some properties. G. FRAENKEL (introduced by H. E. CARTER). *Dept. of Entomology, Univ. of Illinois, Urbana.*

The mealworm, *Tenebrio molitor* requires, in addition to 8 known B-vitamins, a factor present in charcoal filtrates from yeast or liver extract, which has been provisionally named B_T (Fraenkel *et al. Nature, London* 161: 981, 1948). On 'synthetic' diets, without B_T, most larvae die during the 4th–5th week. A testing method has been designed whereby larvae are grown on deficient diets for the first 3 weeks and then transferred to experimental diet. Yeast and liver give optimal effects at levels of 1% (dry weight basis) in the diet. Dry milk is about five times, and dry whey ten times more active. Egg is a poor source, and so are most vegetable materials. B_T is highly water soluble and insoluble in most fat solvents. It is not adsorbed on charcoal, but is by certain brands of Fuller's Earth. In phenol-water systems it is concentrated in the phenol phase. Taking advantage of these properties concentrates have been prepared from whey which gave optimal effects in amounts of 3 μg/gm. of diet, with a loss of approximately 75% of the activity. This amounted to an approximately 333-fold concentration. B_T is remarkably stable to autoclaving in water, heating in acids, and irradiation by ultraviolet light. It appears unlike most, if not all, other unidentified B-factors that have been described for various microorganisms, rats, chicks and guinea pigs. Purified B_T preparations have no strepogenin activ-

ity. B_T is required by one other insect, *Palorus ratzeburgi*.

A natural inhibitor of holophosphorylase. JEROME FREDRICK, IRWIN FEIGIN AND ABNER WOLF (introduced by EDGAR G. MILLER, JR.) *Veterans Administration Hosp., Bronx, Depts. of Pathology and Neurology, Columbia Univ., and Neurological Inst., New York City.*

Holophosphorylase in a simple muscle homogenate is less active than crystalline holophosphorylase derived from an equal weight of homogenate. Fractionation of a muscle homogenate with alcohol containing Ca^{++} yields a crystalline substance which produces complete inhibition of holophosphorylase activity, but does not inhibit phosphorylase activity. The crystals are colorless rhomboid plates which are optically active, soluble in water, slightly soluble in ethanol, and which give a violet ninhydrin color. Paper chromatography shows a single violet spot with R_f values comparable to glutamic acid. The hydrochloride derivative contains 7.2 % nitrogen, shows an optical rotation of + 30°, and decomposes at 201°C. The inhibition caused by the inhibitor is overcome by the addition of 1 % glycogen to the reaction mixture. The formation of a glycogen-inhibitor complex is postulated since inhibitor which is preincubated with glycogen causes no inhibition of holophosphorylase activity. The glycogen, so preincubated, no longer 'primes' the muscle phosphorylase reaction. The 'S' shaped curves reported previously for holophosphorylase in simple homogenates in the presence of 10 mg. % glycogen, can be explained by the progressive synthesis of glycogen resulting in the inactivation of the inhibitor. L-arginine, *dl*-tryptophane, *l*-tyrosine, *dl*-aspartic acid, glutamic acid, *dl*-phenylalanine, and *l*-leucine are inhibitory in 0.01 M concentrations. Glycine, alanine, and histidine are inhibitory in 0.02 M concentrations. Pyruvate, lactate, and acetate are not inhibitory. The inhibition caused by these amino acids is overcome by 1% glycogen. It seems probable that the natural inhibitor is glutamic acid.

Microbial growth inhibition by amino- and nitrophenylalanines. EARL FRIEDEN*, HORACE C. STANSEL, JR.* AND KARL DITTMER. *Dept. of Chemistry, Florida State Univ., Tallahassee.*

A number of substituted phenylalanines in which an amino or a nitro group is substituted for an aromatic hydrogen have been synthesized and studied for their microbiological and certain other properties. In yeast and several strains of *E. coli*, *p*-aminophenylalanine produced a 50% inhibition of growth at 10^{-6} M. This antagonism was nullified by phenylalanine, tyrosine, and tryptophane (see also ELLIOT, D. F., AND C. R. HARRINGTON, *J. Chem. Soc.* 85–89, 1948). *p*-Nitrophenylalanine was a less effective antagonist with similar reversal agents among the natural amino acids. Results with other amino- and nitrophenylalanines will also be presented.

Chemical studies on relaxin. EDWARD H. FRIEDEN. *Biological Labs., Harvard Univ., Cambridge, Mass.*

Relaxin has been found to be a comparatively low molecular weight protein (or polypeptide), sensitive to reducing agents, acetylation, methylation, the action of iodine, and digestion with proteolytic enzymes. Although an appreciable proportion of the total nitrogen of the hormone is free amino nitrogen, nitrous acid does not significantly reduce its activity. Ultracentrifuge and analytical data for preparations of high activity (140 G.P.U./mg.) indicate a probable upper limit of 9000 for the molecular weight. A study of the electrophoretic behavior of relaxin preparations of lower potency (70–90 G.P.U./mg.) suggests the presence of more than one molecular species with relaxin activity. This conclusion is supported by the results of studies of the behavior of relaxin toward precipitating reagents. (Supported in part by a grant from the division of research grants and fellowships, U. S. Public Health Service, to Dr. F. L. Hisaw.)

Desoxyribose-1-phosphate in nucleic acid synthesis. Formation of thymidine. MORRIS FRIEDKIN AND DEWAYNE ROBERTS (introduced by F. EDMUND HUNTER, JR.) *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

The interconversion, desoxyinosine + thymine ⇄ thymidine + hypoxanthine, has been interpreted by Manson (Thesis, Wash. Univ., 1949) to involve a phosphorolysis with desoxyribose-1-phosphate (DRP) as the key intermediate. The availability of crystalline cyclohexylamine DRP (*J. Biol. Chem.* 184: 449, 1950) made possible an investigation of the direct synthesis of thymidine. Seventeen micromoles of thymine and 37 micromoles of DRP were incubated with calf kidney enzyme at 38°. The yield of thymidine after 4 hours was 4.3 micromoles. Separations of thymine and thymidine were accomplished by use of an anion exchange resin column (Dowex-2) developed with 0.01 M ammonium formate buffer, pH 8.9. Absorption spectra in acid and alkaline media were used to characterize eluate fractions. In control experiments where DRP was omitted during incubation yet added after heat inactivation of the enzyme, no thymidine formation could be detected. The average over-all recovery of thymine plus thymidine was 82%. The procedures described offer a technically feasible method of preparing isotopically labeled thymidine of high specific activity. (Supported in part by a (NIH) USPHS research grant.)

Metabolism of 2-carbon acids in the intact rat. BERNICE FRIEDMANN,* H. I. NAKADA* AND S. WEINHOUSE. *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

Various 2-carbon acids labeled with C^{14} were administered to rats by intraperitoneal injection together with sodium benzoate, and their respective conversions to CO_2, oxalate, and glycine were followed. The animals were kept in a closed chamber and respiratory CO_2 was collected by drawing air through the chamber and into CO_2-free alkali. Oxalate was isolated from the urine by the carrier technique, and glycine was recovered from the urine as hippuric acid. Injected oxalate was found to be inert metabolically, being neither oxidized to CO_2 nor reduced to glyoxylate. Glyoxylate was rapidly oxidized to oxalate and was also converted rapidly to glycine. It was oxidized to CO_2 at about the same rate as glycolate and glycine. Glycolate was converted rapidly to glycine, but only slowly to oxalate. Neither acetate nor formate yielded appreciable quantities of oxalate or glycine, though both acids were rapidly oxidized to CO_2. Glycine itself did not yield appreciable quantities of oxalate. The inertness of oxalate indicates that this acid cannot be an intermediate of general metabolic processes. The inability of acetate to yield appreciable quantities of glycine or oxalate apparently eliminates glycolate, glyoxylate or oxalate as intermediates in the oxidation of acetyl groups. The failure of glycolate and glycine to yield large amounts of oxalate is interpreted to indicate that glyoxylate, when formed physiologically, undergoes oxidation to formate and CO_2 rather than to oxalate.

A quantitative study of the acids of the tricarboxylic acid cycle under certain metabolic conditions. CHARLES E. FROHMAN,* JAMES M. ORTEN AND ARTHUR H. SMITH. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

The method of Marshall *et al.* (*J. Biol. Chem.* 179: 1127, 1949) for determining fumaric acid by partition chromatography on silica gel has been expanded to include other acids of the tricarboxylic acid cycle. In the present investigation, the concentrations of fumarate, citrate, isocitrate, cis-aconitate, lactate, alpha-keto-glutarate, oxalacetate, pyruvate, fumarate, succinate, and malate were determined in the liver, brain, kidney, blood, and muscle of normal and experimental animals. No significant differences are found between the levels in normal rats fasted for 24 hours and in those fasted for 72 hours. However, drastic changes in the concentrations of the above acids are found in the tissues of rats made diabetic by an injection of alloxan. In these animals, the concentrations of all the above acids, with the exception of lactate and pyruvate, are decidedly lower. The effect of insulin on the levels of the above acids in the tissues of both diabetic and normal animals has also been determined. Insulin is found to reverse the above effect, increasing the levels of the organic acids toward those of the normal. The foregoing data warrant conclusions as to the possible effect of insulin on the intermediary metabolism of the rat.

Metabolic studies with deuterium steroid hormones. DAVID K. FUKUSHIMA,* KONRAD DOBRINER AND T. F. GALLAGHER. *Sloan-Kettering Inst. for Cancer Research, New York City.*

The relationship of steroid hormones to their urinary metabolites has been investigated in human subjects using compounds stably labelled with deuterium. In an extensive investigation of the fate of testosterone, the hormone as well as many of its metabolites were studied. The principal conclusions drawn were that testosterone is rapidly metabolized and excreted, the major ketonic end products being androsterone and etiocholanolone. No ketonic metabolites were found in the feces. Other steroid metabolites constitute a minor fraction of the total excretion. The ratio of androsterone to etiocholanolone in the urine appeared to be characteristic of the individual. The endogenous production as well as the ratio of androsterone to etiocholanolone was not altered by administration of 100 mg. or 10 mg. of the hormone in a single dose and the total amount recovered was similar. Studies were made with the C_{21} adrenal hormone, 17α-hydroxyprogesterone. The side chain of this compound was lost in part and the resultant C_{19} steroid was metabolized in the same pattern as testosterone. Studies on the metabolic fate of the adrenal hormone, 'Reichstein's Substance S', will also be reported.

The effect of hormonal factors on growth of a transplanted rat tumor. CASIMIR FUNK, PHILIP TOMASHEFSKY,* ROBERT SOUKUP* AND ARTHUR EHRLICH.* *Funk Fndn. for Med. Research, New York City.*

The reduced rate of growth of Walker Carcinosarcoma 256 in hypophysectomized rats is partially counteracted by some anterior pituitary hormones, i.e. growth hormone, thyrotropic hormone and adrenocorticotropic hormone. Other hormones tested but not found to have any marked effect on tumor growth in hypophysectomized rats include: lactogenic hormone, follicle stimulating hormone and pregnant mare serum gonadotropin. In otherwise intact animals, the removal of either the testes or spleen of rats before tumor implantation has a slight accelerating effect on tumor growth, while, the removal of either the adrenals, the thyroid or the ovaries before tumor implantation has a marked inhibiting effect upon tumor growth. When alloxan is given in a dose sufficient

to cause diabetes there is a marked depression in the rate of tumor growth. While no individual hormone, even in relatively large doses, showed a complete effect, the variety of active hormones involved suggests that the control of tumor growth by the pituitary is due to a combination of such hormones and, perhaps, to some non-hormonal factors. The chemistry of the non-hormonal stimulating and inhibiting factors is now being investigated.

Utilization of succinate and α-ketoglutarate for contraction energy by intestinal smooth muscle. ROBERT F. FURCHGOTT AND MARILYN WALES.* *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

If rabbit duodenal segments are suspended in a substrate-free medium and attached to a weighted isotonic lever, a gradual decrease in amplitude of spontaneous contraction of the longitudinal smooth muscle occurs as the endogenous substrate of this muscle is depleted. When the amplitude has fallen to about 10% of the initial level, the percentage recovery of amplitude on adding a substrate to the medium is considered an index of the rate of utilization of the added substrate for contraction energy. Media of pHs from 6.1 to 7.4 (pH adjusted by varying the bicarbonate content and bubbling through 95% 0_2 - 5% CO_2) were used at 37.5°. The lower the pH, the more effective were succinate and α-ketoglutarate in restoring the amplitude of contraction. In each case the effectiveness was proportional to the final concentration of the uni-ionized species of the dicarboxylic acid in the medium. It is proposed that the muscle cells are much more permeable to this species than to the di-ionized species. The results constitute partial evidence for the occurrence of the Krebs cycle in intestinal smooth muscle. In addition to exhibiting a marked substrate effect at low pHs, high concentrations of succinate and ketoglutarate (.01 *M* or higher) as well as certain monocarboxylic acids produced a temporary inhibition of contractile activity of intestinal smooth muscle shortly after being added. (Supported in part by a grant from the Life Insurance Med. Research Fund.)

Conjugation of benzoic and phenylacetic acids with glycine and glucuronic acid during induced growth. O. H. GAEBLER, W. T. BEHER* AND E. M. CRIGGER.* *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

It is well known that, in the dog, hippuric acid and glucuronic acid monobenzoate are both produced in quantity from ingested benzoate. The two metabolic pathways involve formation of peptid and ester linkages respectively. Metabolism of phenylacetate also follows both routes, but it is claimed that, in the dog, conjugation of phenylacetate occurs in the liver while that of hippuric acid takes place in the kidney. It appeared of interest to determine whether or not administration of growth hormone or testosterone propionate, both of which produce nitrogen storage, would increase the peptid linked fraction of these acids at the expense of the esterified fraction. Accordingly, the ratio of amounts conjugated with glycine and glucuronic acid respectively was studied in 8 experiments, each of which lasted over 25 days. Four bitches were used. There were 4 experiments with testosterone propionate. Metabolism of benzoate was studied in 2 of these and that of phenylacetate in the other 2. In the remaining 4 experiments, the effect of various doses of growth hormone on metabolism of benzoate was investigated. In all 8 experiments the anticipated nitrogen storage was produced, but the ratio of the amount of either acid conjugated with glycine to that conjugated with glucuronic acid was not significantly altered. No evidence was therefore obtained that either hormone promotes formation of peptid linkages. The balance between the alternative routes of metabolism varied greatly in different animals. Other factors which might account for this variation are being studied.

Microbiological activity of cortisone acetate. DOROTHY SUE GAINES AND HARRY P. BROQUIST (introduced by THOMAS H. JUKES). *Lederle Labs. Div., American Cyanamid Co., Pearl River, N.Y.*

Dehydroisoandrosterone acetate was found to have pteroylglutamic-acid-like activity for *Streptococcus faecalis* R and *Lactobacillus casei* (Gaines, D. S. and J. R. Totter, *Proc. Soc. Exper. Biol. & Med.* 74: 558, 1950). It was found that a clinical preparation of cortisone acetate supported growth of *S. faecalis* R in the absence of pteroylglutamic acid and growth of *Leuconostoc citrovorum* 8081 in the absence of citrovorum factor (CF). Cortisone acetate at levels of 250 to 500 μg/ml. of culture medium was found to give half-maximum growth at 16 hours with *Le. citrovorum*. Evidence that contamination with CF was not responsible for this growth was obtained by steaming the cortisone preparation in dilute HCl at pH 2 for 30 minutes. This treatment markedly increased the activity of the cortisone preparation for *Le. citrovorum*, whereas CF is almost completely destroyed by such a procedure. The acid-treated cortisone preparation was found to reverse the inhibitory effect of 4-aminopteroylglutamic acid upon the growth of *Le. citrovorum*. Mice were given subcutaneous injections of 1 mg. of cortisone acetate and 10 μg. of 4-aminopteroylglutamic acid three times weekly. The cortisone appeared to increase the toxicity of the 4-aminopteroylglutamic acid.

The degradation of hemoglobin by tissue extracts and by ascorbic acid. R. L. GARNER,* GORDON C. MILLS* AND A. A. CHRISTMAN. *Dept. of Biological Chemistry, School of Medicine, Univ. of Michigan, Ann Arbor.*

It has been reported that extracts of certain tissues can convert hemoglobin into green pigments. The reaction is not well characterized but it has been assumed to be an enzymatic process. A detailed study of a rat liver tissue—hemoglobin system has shown that the degradation of the hemoglobin is due to the presence of a heat stable, readily diffusible, non-protein substance. This material has been tentatively identified as ascorbic acid. The product of the reaction is *choleglobin* which, upon treatment with acid, is converted into *biliverdin*. Solutions of pure ascorbic acid in concentrations which approximate those of the apparent total ascorbic acid in liver extracts readily convert hemoglobin into choleglobin. The rate of conversion of hemoglobin into choleglobin by ascorbic acid is accelerated by glutathione. When an extract of red cells is added to the above system a marked dimunition of the rate of choleglobin is observed. The material obtained from the red cells has been termed the *erythrocyte factor* and it exerts its inhibitory action only in the presence of glutathione. The chemical nature of this factor is under investigation. These findings do not imply that the *in vivo* conversion of hemoglobin into bile pigment is necessarily non-enzymatic; they do suggest that investigations of the biological degradation of hemoglobin must take into consideration the ascorbic acid content of the tissue preparations.

Urinary excretion of thiosulfate by adult rats. JOSEPH H. GAST AND KAZKO ARAI (introduced by C. R. TREADWELL). *Dept. of Biochemistry, Baylor Univ. College of Medicine, Houston, Tex.*

Mature albino rats of the Sprague-Dawley strain were placed in metabolism cages, fed weighed portions of powdered, moistened Purina Dog Chow and supplied water *ad libitum*. Urines were collected for a 48-hour period and preserved with chloroform. Average values for 6 animals of each sex calculated on the 24-hour basis were as follows: males excreted 429 mg. total nitrogen, 19 mg. total sulfur and 37 μg. of thiosulfate sulfur while females excreted 437 mg. of total nitrogen, 22 mg. of total sulfur and 63 μg. of thiosulfate sulfur. The daily excretions of thiosulfate sulfur ranged from 31–45 μg. for males and 39–82 μg. for females per 24-hour period. The nitrogen to sulfur and total sulfur to thiosulfate-sulfur ratios in this period were higher than we had previously observed in humans. In additional periods of observation the range of daily excretion of thiosulfate sulfur for individual animals was from 19–71 μg. for males and 32–141 μg. for females.

Aluminum oxide adsorption and corticosteroids. JACQUES GENEST (introduced by REGINALD M. ARCHIBALD). *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

When subjected to Archibald's chromatographic procedure for steroid fractionation (*Federation Proc.* March 1949; April, 1950), untreated and, as furnished to us, corticosterone (B), dehydrocorticosterone acetate (A), 11-desoxycorticosterone (DOC), 17-hydroxycorticosterone (F), 11-dehydro-17-hydroxycorticosterone (E) and 11-desoxy-17-hydroxycorticosterone acetate (S) were eluted in major part by water. The fractions eluted with water gave, in each case, the same dark brown color with the Zimmerman Reagent as the compounds before chromatography. Except for DOC, a much smaller component is eluted earlier by 0.5 or 2% ethanol in benzene and this component from E, F and S gives with the Zimmerman Reagent a pink color typical of the 17-ketosteroids. As measured by ultraviolet spectrophotemetry and the *m*-dinitrobenzene reaction, 15 to 50 % loss occurred in the chromatographic procedure except for DOC (recovered 100 %). If the above corticosteroids are refluxed with one-tenth vol. concentrated HCl for 30 minutes prior to the chromatographic procedure, most of the product is still eluted by water. By Corcoran and Page's method for measurement of formaldehydogenic substances only 5–15 % of the initial amount of the unrefluxed corticosteroids, were accounted for in the water eluates after chromatography on Al_2O_3. The absorption curves (210–750 mμ) of the products resulting from treatment of the six corticosteroids with concentrated H_2SO_4 was altered markedly by prior adsorption of the corticosteroids on Al_2O_3 and to a slightly lesser extent by refluxing with one-tenth vol. concentrated HCl for 30 minutes.

Quantitative studies of protein and nucleic acid synthesis in tissue culture. HORACE W. GERARDE,* MARION JONES,* SHIH C. WANG,* DONALD F. WILSON* AND THEODORE WINNICK. *Univ. of Iowa, College of Medicine, Iowa City.*

Methods have been developed for cultivating 20–40 mg. quantities of minced embryonic chick heart, lung, and intestine in roller tubes. The initial protein and nucleic acid content of each tube was calculated from the wet weight of the tissue and the previously established ratio of protein and nucleic acid concentration to wet weight. After varying times of incubation, tubes were removed and their protein content determined by direct isolation and weighing. Pentose nucleic acid (PNA and desoxypentose nucleic acid (DNA)

analyses were made on aliquots of this isolated material. Extensive net protein synthesis resulted with tissues grown in 25% embryo extract, but not with the synthetic media tested. After 2-week incubation, the protein content had doubled with heart and intestine, quadrupled with lung. The protein at the 2-week stage had the same % nitrogen and gave the same intensity of ninhydrin and biuret colors as the zero time protein. When C^{14}-labeled glycine, alanine, or phenylalanine was present in the nutrient media, extensive C^{14} incorporation into protein occurred. By subjecting hydrolyzed radioactive proteins to Moore-Stein starch column chromatography, it was found that the labeled amino acids comprised relatively high percentages of the total glycine, alanine, and phenylalanine residues of the proteins. With lung a doubling of the quantity of PNA per culture tube resulted in 2 weeks, but no net DNA synthesis.

Adenylic acid deaminase activity of myosin. JOHN GERGELY (introduced by L. J. TEPLY). *Natl. Insts. of Health, Bethesda, Md.*

Repeatedly precipitated myosin preparations show 5-adenylic acid deaminase (AAD) activity. Recently the identity of AAD and myosin has been suggested (HERMANN AND JOSEPOVITZ. *Nature, London* 164: 847, 1949). It has been demonstrated that the adenosine-triphosphatase (ATP-ase) activity of myosin does not depend on the integrity of the myosin molecule (GERGELY. *Federation Proc.* 9: 176, 1950). It seemed therefore of interest to clarify also the relationship between AAD and myosin. The AAD activity per mg. of protein of myosin is about 1/20 of the activity of purified AAD, when tested at the same substrate level. K_m is of the order of 5×10^{-4} M both for purified AAD and myosin. Serum albumin has an activating effect on the purified AAD, but does not affect myosin. Trypsin-treated myosin preparations retain their AAD activity, as they retain their ATP-ase activity. Our observations seem to rule out the identity, but suggest a close association of myosin and the enzyme AAD.

Enzymatic oxidation of molecular hydrogen by bacterial extracts. HOWARD GEST (introduced by L. O. KRAMPITZ). *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Cell-free preparations which catalyze the oxidation of H_2 in the dark have been obtained from the photosynthetic bacterium *Rhodospirillum rubrum*. The only suitable hydrogen acceptor found thus far is methylene blue; although intact cells of R. rubrum rapidly oxidize H_2 in the presence of methylene blue and $K_3Fe(CN)_6$, the latter is not readily reduced by cell-free preparations. The enzyme (hydrogenase) is rapidly inactivated by air but can be preserved by storage under H_2 at 5° C. (also found for the hydrogenase of *E. coli* by BACK *et al. Aust. J. Sc.* 9: 25, 1946). The rate of H_2 oxidation by crude extracts is not affected by metal ion complexing agents such as $\alpha\ \alpha'$-dipyridyl and diethyldithiocarbamate. Participation of easily dissociable organic coenzymes in the hydrogen transfer is unlikely since there is no appreciable loss in activity upon anaerobic dialysis against water saturated with H_2. A short but definite lag period has been noted in the H_2 consumption by dialyzed preparations. The lack of inhibition by 0.001 M *p*-chloromercuribenzoate suggests that the inactivation by air is not attributable to oxidation of essential sulfhydryl groups. It has been proposed previously that this inactivation is due to oxidation of a ferrous porphyrin prosthetic group to the inactive ferric form. In order to obtain direct evidence concerning the nature of the prosthetic group, the isolation of the enzyme from sonic extracts is in progress. Partial purification has been achieved by a procedure employing freezing and thawing, $MnCl_2$ treatment, and $(NH_4)_2SO_4$ fractionation.

The effect of methionine sulfoximine on different species of animals. S. N. GERSHOFF* AND C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Methionine sulfoximine isolated from nitrogen trichloride treated zein and flour has been established as the causative agent of 'running fits' in dogs. Through the courtesy of Dr. L. Reiner, Wallace and Tiernan Co., Inc., Newark, N. J., this compound has been made available to us for experimental purposes. We have determined that the amount of methionine sulfoximine necessary to produce 'running fits' in dogs in 3–5 mg./kg. of body weight. Monkeys, previously reported resistant to nitrogen trichloride treated proteins have been brought down with typical seizures by injecting them with approximately one-hundred times the amount of methionine sulfoximine needed to produce this condition in dogs. Abnormal electroencephalograms and severe convulsions have been observed in these monkeys. These effects of methionine sulfoximine have been prevented by the administration of methionine. The anti-methionine activity of methionine sulfoximine has also been demonstrated in rats by studies involving liver xanthine oxidase activity. Detoxification studies using the organs of various animal species have been made in order to determine whether differences in sensitivity to methionine sulfoximine are due to detoxification mechanisms present in some species and absent in others.

Extrahepatic lipid oxidation by the rat. R. P. GEYER, W. R. WADDELL, J. PENDERGAST AND

G. YEE (introduced by F. J. STARE). *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Radioactive pentanoic and octanoic acids were injected intravenously, intraperitoneally, or subcutaneously into normal, partially hepatectomized or eviscerated rats. The absolute rate of conversion of the substrates to $C^{14}O_2$ was somewhat below normal in the case of the operated animals, whereas the rate based on specific activity was normal or above. Seventy to ninety % of the C^{14} appeared as $C^{14}O_2$ in 4 hours. Similar results were obtained with palmitic acid ($-C^{14}OO-$) dissolved in olive oil which was administered intravenously in a highly emulsified form. Approximately 60% of the radiocarbon was converted into $C^{14}O_2$ in 20 hours. When tripalmitin was substituted for palmitic acid, the absolute rate of conversion and the rate based on specific activities were below normal in the case of the eviscerated rats. The results show that free fatty acids are rapidly oxidized by non-hepatic tissues of the rat *in vivo*.

Isolation of adrenocorticotrophin (ACTH) and thyrotrophin (TSH) from hog pituitary tissue. B. N. GHOSH,* DIXON M. WOODBURY,* GEORGE SAYERS* AND EMIL L. SMITH. *Dept. of Pharmacology and Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.*

A new method has been developed for the assay of TSH. The method is based on the ability of TSH to increase the uptake of I^{131} by the thyroid of the hypophysectomized rat. The log-dose response curve and the statistics of the bioassay procedure will be presented. The new method of assay of TSH and the adrenal ascorbic acid-depletion method of assay of ACTH have been applied to the isolation of these hormones from hog pituitary tissue. Ten gm. of dry hog pituitary tissue are ground and extracted with 500 ml. of 80% acetone at pH 8.0. Two gm. of LiCl are dissolved in the supernatant, which contains all the ACTH activity of the glands, and the solution is adjusted to pH 5.4 with LiOH. The precipitate contains no more than 5% of the total ACTH activity of the extract. Three volumes of acetone are mixed with the supernatant. The precipitate weighs 700–900 mg. and is about twice as potent as La-1-A (ACTH standard) and ten times as potent as dried hog pituitary tissue. The method gives an almost quantitative recovery of ACTH from pituitary glands. The residue which remains after extraction of pituitary tissue with 80% acid-acetone contains practically all the TSH activity. This residue is extracted with aqueous NaOH at pH 10.0; the pH is lowered to 4.0 and the precipitate discarded. The supernatant contains the TSH activity. Additional steps in purification of TSH will be described.

A new mechanism of ethanol formation in the heterolactic fermentation. MARTIN GIBBS AND R. D. DEMOSS (introduced by I. C. GUNSALUS). *Brookhaven Natl. Lab., Upton, L. I., N. Y.; Indiana Univ., Bloomington.*

Fermentation of glucose by the heterofermentative coccus, *Leuconostoc mesenteroides*, yields lactate, ethanol and CO_2 in equimolar ratio. The rate is increased by acid reaction or by added acetate, acetaldehyde, acetoin or diacetyl. Dried cell preparations produce one mole of lactate per mole of glucose, or HDP only, therefore accounting for but half of the carbon of the substrate. Cell-free extracts contain aldolase and DPN linked dehydrogenases for triose phosphate, lactate, ethanol and butylene glycol. These experiments indicate: 1) Formation of lactate via the usual Meyerhof-Embden scheme, 2) lack of mixing of carbon skeletons at the triose stage, 3) formation of ethanol and CO_2 by an undisclosed mechanism. To test these possibilities C^{14} glucose was fermented with cell suspensions, using conditions which yield the products indicated. Glucose 1-C^{14} yielded $C^{14}O_2$, ethanol with a trace of C^{14}, and unlabelled lactate. Glucose 3,-C^{14} yielded half of the C^{14} in the carboxyl of lactate, and half in the carbinol of ethanol, with a trace in the CO_2. With a mixture of carboxyl labelled acetate and unlabelled glucose,C^{14} was found exclusively in the carbinol of ethanol. Thus the isotope experiments confirm the conclusion that a new pathway for ethanol formation exists. (Research sponsored in part by Atomic Energy Commission.)

Synthesis of phenylalanine and tyrosine in yeast. C. GILVARG* AND K. BLOCH. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Yeast was grown on a medium containing both glucose and acetate, one of which was labeled by C^{14}. The isotope distribution in the amino acids of the yeast protein was investigated. The data for phenylalanine and tyrosine indicate that the benzene rings and the side chains of these two amino acids are derived from a six-carbon unit and a three-carbon unit respectively, and that these precursors are formed rather directly from glucose. Members of the tricarboxylic acid cycle are excluded as intermediates. *meso*-Inositol, a possible intermediate in the formation of benzene rings from glucose, prepared biosynthetically from $C^{14}O_2$, was not utilized in the synthesis of tyrosine and phenylalanine by yeast. When 1-C^{14} glucose was the labeled substrate, only the β-carbon of the amino acid side chains contained C^{14}, while little isotopic carbon was present in any of the side chain carbons when the culture medium contained

non-isotopic glucose and C^{14} acetate. This result points to a three-carbon intermediate of glycolysis as the precursor of the side chains. In the experiments with labeled acetate, the amino acids serine and glycine, in contrast to the dicarboxylic acids, alanine, lysine, leucine and valine, contained very little C^{14}. Serine and glycine, like the side chains of phenylalanine and tyrosine, appear to be formed from a three-carbon intermediate of glycolysis.

Observations on the simultaneous metabolism of acetate and carbon dioxide by resting cell suspensions of *Rhodospirillum rubrum*. J. GLOVER* AND M. D. KAMEN. *Washington Univ. Med. School, St. Louis, Mo.*

The light and dark metabolism of *R. rubrum* in phosphate-buffered media containing *a*) 2-C^{14}-acetate and bicarbonate, *b*) C^{14}-bicarbonate and acetate *c*) C^{14}-bicarbonate and H_2, have been studied by radioautography of 2-dimensional papergrams prepared from non-volatile water-alcohol soluble and insoluble fractions obtained after labeling periods of from 2 seconds to several hours. The labeling pattern in *a*) differs markedly from those in *b* and *c*. In *a* there is rapid incorporation of acetate carbon into lipid, as much as 30–50% appearing in this fraction in 30 seconds. By maintaining the temperature at 0°C. and the labeling period less than 10 seconds, more than 90% of the labeled carbon is found to remain in the water-soluble, ether-soluble fraction. The most intense labeling under these conditions occurs in a compound (s) which does not show R_f values characteristic of any of the conventional Krebs-cycle components, phosphate esters, or amino acids. On the other hand, photo-reduction as in *c* results in much slower appearance of label in insoluble material, particularly lipid, and the major labeling appears to be in phosphate esters, and, to a lesser extent, in dicarboxylic acids, (succinic, malic etc.). In *b*, pickup of carbon is very much lower than in *c* indicating repression of CO_2 fixation by acetate. The labeling pattern in *b* resembles that obtained in *c*. Dark metabolism in *a* results primarily in labeling of a few unknown substances as well as Krebs-cycle compounds. In *c* dark metabolism resembles light metabolism, the level of fixation being lower by an order of magnitude, however. In *b* practically no dark pickup occurs, but what little labeling is observed appears to show R_f values of phosphate esters. These results indicate that short-term photometabolism of acetate and CO_2 by *R. rubrum* in the resting state does not involve reactions between acetate and CO_2 or metabolic fragments derived therefrom. The bearing of these results on the general mechanism of photosynthesis will be discussed.

Effect of aminopterin on nucleic acid metabolism in the rat. DAVID A. GOLDTHWAIT* AND AARON BENDICH. *Sloan-Kettering Inst. for Cancer Research, New York City.*

The effect of aminopterin in decreasing the incorporation of formate into nucleic acids has been confirmed. In addition it was found that, following the administration of a single dose of aminopterin which leads to death in 4 days, the ability to synthesize nucleic acids is regained partially prior to death. When C^{14}-formate and aminopterin were administered simultaneously and the animals were sacrificed after 24 hours, the C^{14}-activity of the total nucleic acids was 30% of the control value, and when formate was administered 24 or 48 hours later the values were 35 or 71% respectively. It was found that, without aminopterin, increasing levels of adenine resulted in decreasing incorporation of formate into total nucleic acids, suggesting that the availability of adenine spares the biosynthesis from formate. When both C^{14}-formate and N^{15}-adenine were administered, aminopterin decreased the incorporation of formate into the intestinal DNA adenine, guanine, and thymine to 6–8% of the control levels, while decreasing that into PNA adenine and guanine to only 32% of the control values.

Chemical and isotopic tracer studies of human serum lipoproteins with the aid of the quantity ultracentrifuge. WILLIAM H. GOLDWATER, MALCOLM L. RANDOLPH, J. R. SNAVELY AND ROY H. TURNER (introduced by WILLIAM B. WENDEL). *Depts. of Medicine and Biophysics, Tulane Univ. School of Medicine, New Orleans, La.*

Quantity ultracentrifugation of human serum results in the redistribution of serum lipids into separate compartments, presumably due to the stratification of lipoproteins by sedimentation and density effects. A 'cream' zone at the top of the tube contains all lipids, predominantly neutral fat. Near 1.020 density are found peak concentrations of all lipids. Indications of other lipid accumulations occur in the regions of 1.035, 1.055, and the bottom of the tube, with free cholesterol absent at densities higher than 1.035, and cholesterol esters minimal at the bottom. A neutral fat peak appears near 1.010 in some sera. That these compartments contain distinct entities seems indicated by their differing chemical compositions. Radioactive phosphorus, P^{32}, has been administered to subjects to label the phospholipid components. The different rates of isotope uptake by lipid phosphorus at various density levels give added support to the proposition that different lipoproteins with presumably separate metabolic

roles, at least with respect to the phospholipid fractions, are concentrated at the various levels.

Origin of plasma cholesterol using carbon^{14}. R. GORDON GOULD, DONALD J. CAMPBELL,* C. BRUCE TAYLOR,* FRANK B. KELLY, JR.,* IRVING WARNER* AND CARL B. DAVIS, JR.* *Rush Depts. of Biochemistry, Pathology and Surgery, Presbyterian Hosp., Chicago, Ill.*

It is now well established that liver and many other tissues are capable of cholesterol synthesis but relationships between tissue and plasma cholesterol are obscure. Evidence strongly favoring the liver as the source of plasma cholesterol in the dog has been obtained by intravenous administration of labeled acetate to sham-operated control and hepatectomized dogs. Although acetate metabolism was essentially normal in the latter as judged by its oxidation to CO_2 and conversion to cholesterol in various tissues, very little isotopic cholesterol had appeared in plasma 4 hours after acetate administration. In control dogs, the specific activity of bile cholesterol was invariably higher than that of any tissue studied. The plasma values were about thirty times as high as in the hepatectomized animals and equal to or somewhat lower than those of liver. The high bile value suggests synthesis in liver and immediate secretion in bile without complete mixing with all hepatic cholesterol. The relatively high value reached by plasma in this short time may be explained either on a similar basis as for bile or on the assumption of an equilibrium between liver and plasma cholesterol. This second possibility has been explored by injecting blood containing labeled lipoproteins into a normal dog and then following the appearance of labeled cholesterol in liver and its disappearance from plasma. Preliminary results indicate that equilibration is about two-thirds complete in 4 hours and, hence, favor the second hypothesis.

Effect of vitamin B^{12}, *dl*-methionine and vitamin E on the growth promoting value of *Torula* yeast protein. JOSÉ A. GOYCO (introduced by CONRADO F. ASENJO). *Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Puerto Rico, San Juan.*

Male Wistar rats 28 days old were used. The basal diet contained 18% protein supplied by *Torula* yeast. Trials lasted 4 weeks. Growth promoting value of *Torula* yeast was 1.23. When this ration was supplemented with 200 μg. B_{12} per kg. the value was 1.40. The difference was found to be not significant. The incidence of hepatic necrosis in both groups was very high. In a group of animals supplemented with the same amount of B_{12} and 2 mg. vitamin E per week given separately, the growth promoting value was significant, 1.63. No hepatic necrosis was observed in these rats. In another experiment the same basal ration was used except that it contained 0.5% methionine. One group received this ration only, a second group the ration separately supplemented with one μg. B_{12} per day, and a third group the ration separately supplemented with one μg. B_{12} per day and 2 mg. vitamin E per week. Growth promoting values were: 2.01, 2.54 and 2.77, respectively. The difference between those on methionine alone and those receiving in addition B_{12} was significant. Not a single one developed hepatic necrosis. B_{12} as well as methionine increased food consumption to about the same extent. When fed together the increase in food intake was almost additive. Methionine, B_{12} and vitamin E together step up the growth promoting value of *Torula* yeast protein to 2.77, a level never observed by us before.

A method for the ultracentrifugal analysis of α and β serum lipoproteins. ARDA ALDEN GREEN, LENA A. LEWIS* AND IRVINE H. PAGE. *Research Div., Cleveland Clinic Fndn., Cleveland, Ohio.*

The density of human sera has been increased to 1.21 by the addition of potassium bromide or of potassium bromide and 9.4% sodium chloride. Under these conditions no serum proteins precipitate and α as well as β lipoproteins have a negative sedimentation velocity. After preliminary concentration, the pattern in the analytical ultracentrifuge was determined. The identity of the fractions is being studied by electrophoretic, chemical and ultracentrifugal methods.

Mechanism of antibody synthesis. HOWARD GREEN* AND H. S. ANKER. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Studies on turnover rates of antibody protein by Schoenheimer, Heidelberger *et al.* (*J. Biol. Chem.* 144: 545, 1942), using labeled glycine, have emphasized the chemical similarities previously shown to exist between antibodies and serum globulins. These similarities suggest that the two proteins either have a common origin or that the serum globulin (or a similar protein) may be a specific precursor of antibody. An experiment was performed in an attempt to decide between these alternatives. A labeled amino acid was injected into a rabbit and a period of time allowed to elapse during which most of the isotope was incorporated into body protein, while the isotope concentration in the amino acid pool fell to a rather low level. Injections of egg albumin antigen were now given, and the isotope concentration determined in the resulting antibodies, in plasma proteins and in the amino acid pool. It was found that the isotope

concentration in the antibodies exceeded that of the free amino acid during the period over which the antibody was synthesized, indicating that the synthesis occurred at least in part from sources other than free amino acids. The isotope concentration of various serum protein fractions was sufficiently high so that these could have served as precursors of the antibody. Further experiments are being performed in order to determine whether cellular protein can be excluded as the antibody source.

Synthesis of purine in dialyzed liver extracts. G. ROBERT GREENBERG (introduced by HARLAND G. WOOD). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Employing carbon-labeled formate, glycine, and CO_2 it has been shown that hypoxanthine is formed in pigeon liver preparations by reactions involving inosine-5-phosphate as an intermediate (*Federation Proc.* 9: 179, 1950; and in press). The overall reaction has been formulated as follows: 3 NH_3 + glycine + CO_2 + 2 formate + ribose-1-PO_4 → inosine-5-phosphate $\xrightarrow{-H_3PO_4}$ inosine $\xrightarrow{+H_3PO_4}$ hypoxanthine + ribose-1-PO_4. Complete synthesis of purine has now been obtained in dialyzed extracts of pigeon liver acetone powder under anaerobic conditions. Inosine-5-PO_4 tends to accumulate in these extracts apparently because of the partial removal of phosphatase activity. Besides glycine, bicarbonate and formate the following factors appear to be necessary for optimal synthesis; glutamic acid or glutamine, a source of ribose-1-phosphate, and boiled liver extract. Preliminary evidence indicates that the boiled extract may be replaced by a combination of biotin and citrovorum factor. A coupled source of energy, in this case provided by the addition of the constituents for glycolysis, is also required. Studies are in progress to isolate intermediate compounds in the reaction. With whole homogenates both C^{14}-formate and C^{14}-formaldehyde were incorporated into hypoxanthine. Dilution studies show that the incorporation of these two compounds does not necessarily involve their direct interconversion but suggest rather that they are converted to a common intermediate.

Biotin-like activity of octadecenoic acids as affected by positional- and stero-isomerization. SAMUEL M. GREENBERG,* AMBER L. S. CHENG,* DANIEL MELNICK AND HARRY J. DEUEL, JR. *Dept. of Biochemistry, School of Medicine, Univ. of Southern California, Los Angeles and The Best Foods, Inc., Bayonne, N. J.*

A number of positional- and stero-isomeric octadecenoic acids and mixtures of fatty acids derived from natural and hydrogenated vegetable oils have been tested by multiple-level assays for biotin-like activity using *Lactobacillus arabinosus*. The *cis*-octadecenoic acids tested give no great differences in biotin-like activity; however, wide variations are revealed by the *trans* acids. *Trans*-9-octadecenoic (elaidic) acid possesses the greatest activity among the *trans* acids tested and is equal to that of *cis*-9-octadecenoic (oleic) acid. Activity of the *trans* acids tested decrease for positional change of the double bond on either side of *trans*-9- to almost no activity for *trans*-6- and for Δ-17-octadecenoic acids. Assay of the acids in combination with biotin reveal only additive effects for the *cis* compounds tested but considerable synergistic activity for the *trans* compounds except for *trans*-9-octadecenoic acid. Iso-oleic acid mixtures formed by hydrogenation of vegetable oils and the fatty acids from the limpid vegetable oils exhibit a biotin-like effect comparable to that of oleic acid itself. Saturated fatty acids show no biotin-like activity; however, the saturated acids augment the activity of the unsaturated fatty acids in the mixture. An equation has been evolved to permit calculation of expected biotin-like activity of fatty acids of natural and hydrogenated oils from their fatty acid compositions.

Effect of cysteine in reducing the toxicity of nitrogen mustards. A. CLARK GRIFFIN AND EUGENIA L. BRANDT.* *Dept. of Chemistry, Stanford Univ., Stanford, Calif.*

The nitrogen mustards, methyl-bis(β chloroethylamine hydrochloride (HN2) and tris(β chloroethyl)amine hydrochloride (HN3) induce many cellular changes. These agents are highly mutagenic, carcinogenic, they result in metabolic abnormalities in embryonic tissues, and they have been employed in the treatment of leukemia. Actually the nitrogen mustards closely resemble x-radiation in their effects on living cells. Recently Patt and associates (*Proc. Soc. Exper. Biol. & Med.* 73: 18, 1950) observed that administration of cysteine protected animals subsequently exposed to otherwise lethal doses of x-radiation. The similarity in action of the nitrogen mustards and x-ray made it appear probable that cysteine would reduce the toxicity of the nitrogen mustards. In the present study it was found that cysteine, administered intraperitoneally (1 gm./kg. body weight), 5–45 minutes before injection of nitrogen mustards in albino rats, swiss or dba mice, permitted the safe administration of six to eight times the normal dose of HN2. In the studies completed thus far this protective effect has been found to be specific for cysteine. Methionine, cystine, British anti-Lewesite, thioglycollic acid, methylene blue, choline, ascorbic acid, and glutathione were without effect at the levels

administered. The cysteine did not result in a complete inactivation *in vivo* of the subsequently administered nitrogen mustard since several of the characteristic properties of the latter compound appeared to be retained. This cysteine protection may provide a partial explanation as to the mechanism involved in nitrogen mustard-cellular reactions.

Enzymatic mechanism of the biosynthesis of citrulline. S. GRISOLIA* AND P. P. COHEN. *Lab. of Physiological Chemistry, Univ. of Wisconsin, Madison.*

Studies in this laboratory with a partially purified enzyme preparation from rat liver (prepared by differential centrifugation, acetone and alcohol fractionation) have confirmed the requirements of carbamylglutamate for the enzymatic synthesis of citrulline from ornithine. It has now been possible to demonstrate the catalytic role of carbamylglutamate. Up to 10 equivalents of citrulline can be synthesized per mole of carbamylglutamate under suitable conditions. The saturation level for carbamylglutamate is of the order of 2×10^{-4}M. Isotopic studies with $C^{14}O_2$, $N^{15}H_3$ and carbamylglutamate labelled with C^{14} in the carbamyl carbon have shown that carbamylglutamate does not react by transfer of the carbamyl group to ornithine. The partially purified preparation from rat liver has been further resolved into two enzymatic systems: one thermolabile and the other relatively thermostable. Since the latter is completely inactive with carbamylglutamate an insight into the mechanism of the reaction has been obtained. The first system catalyzes the reaction of carbamylglutamate with CO_2 and NH_3 in the presence of Mg^{++} ions and ATP to form an intermediate compound. The second system forms citrulline only upon the addition of the neutralized deproteinized mixture of the first incubation system, containing the intermediate, and ornithine. The first reaction has been followed by the liberation of inorganic phosphate in the medium and $C^{14}O_2$ fixation. The second reaction has been followed by measuring citrulline synthesis. Some properties of the intermediate will be presented.

Protein SH and S-S groups by potentiometric silver titration. BENJAMIN GRUNBAUM* AND EDWARD L. DUGGAN (introduced by DAVID M. GREENBERG). *Dept. of Biochemistry, Univ. of California Med. School, Berkeley.*

A potentiometric method of titration for SH and S-S groups in proteins has been devised. The method consists of a titration with dilute silver nitrate under conditions similar to those of the amperometric titrations, pH 10 in presence of ammonia. The end point is obtained from the maximum slope of the potential vs. volume curve. Titration of 0.2 ml. samples is possible, an 85 λ burette being used. The limit of the method is about 0.02 microequivalents SH with precision of ± 5%. Greater precision is possible with larger quantities. Cysteine hydrochloride is used as the standard. Results indicate that SH groups are absent in insulin, ribonuclease, pepsin and preparations of ACTH protein or peptide. Bovine albumin yielded 0.62 groups per mole, in confirmation of others. Tobacco mosaic virus yielded 0.3% cysteine, increasing on denaturation with dodecyl sulfate to 1.1%. Following peptic digestion and sulfite cleavage of S-S bonds, certain proteins yielded S-S groups by titration in agreement with microbiological assay. ACTH protein S-S groups were incompletely titrated after enzymatic digestions and sulfite cleavage. Three groups were obtained by titration for a preparation which yielded 6–7 groups by assay. The ACTH peptide S-S groups were completely titratable. Both protein and peptide preparations were active by ACTH assay after sulfite treatment and titration. See also the abstract of I. Harris and C. H. Li.

Acetylation of sulfur amino acids by liver and kidney slices. HELMUT R. GUTMANN* AND JOHN L. WOOD. *Div. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis.*

Rat liver and rat kidney slices were incubated with *p*-bromophenyl-L-cysteine or *S*-benzyl-DL-homocysteine labeled with S^{35}. After 4–5 hours 25–40% of the radioactive sulfur compounds in the incubation mixtures were converted into chloroform-soluble products. Liver or kidney homogenates, upon incubation with these substrates, yielded only very small amounts of chloroform-soluble sulfur compounds. *N*-acetyl-*S*-benzylhomocysteine was identified in the chloroform-extract of the appropriate incubation mixture by addition of inactive carrier and subsequent isolation of the compound labeled with radioactivity. *N*-acetyl-*S*-benzylhomocysteine was converted to *S*-benzylhomocysteine by acid hydrolysis without a change in the specific radioactivity of the sulfur. The specific radioactivity of the isolated material indicated that approximately 40% of the chloroform-soluble sulfur was accounted for as *N*-acetyl-*S*-benzylhomocysteine. The data provide experimental evidence that the acetylation of certain sulfur amino acids takes place both in liver and kidney of the rat.

Purification of renin and production of antirenin in man. ERWIN HAAS, HILDEGARD LAMFROM,* AND HARRY GOLDBLATT.* *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.*

A procedure has been designed permitting the isolation of highly purified renin on a pilot plant

scale. Fresh kidneys in lots of 150 pounds can be processed and time-consuming and expensive steps of previous methods, such as grinding of tissue with abrasives, separation of medulla and cortex, defatting and drying, have been omitted in the new procedure. The renin is obtained in the first crude extract in a yield of 3–5 dog units per gm. of tissue and after that it is purified 13,000-fold in 9 subsequent steps of fractionation. The final product, as tested in dogs, has an activity of 190 units per mg. of protein and it is 92 % pure, on the basis of solubility determinations. Repeated subcutaneous injection of renin obtained from hog kidneys into human hypertensive patients has thus become possible, resulting in the development of antirenin in titers as high as 38 units per cc. of serum. The details of this work are to be reported later. The renin preparations, after step #6 of the isolation procedure, can safely be administered to human patients. Emphasis has been placed on the complete removal of anaphylactoid and depressor substances, to avoid the undesirable side reactions which are so frequently encountered following injection of foreign proteins.

Factors required for growth of the Donovan body (*D. granulomatis*). W. Knowlton Hall, Calvin H. Chen* and Robert B. Dienst.* *Depts. of Biochemistry and Microbiology, Med. College of Georgia, Augusta.*

Investigation of the factors contained in egg yolk which are required by the Donovan body for growth in the medium used by Dienst, Greenblatt and Chen (*Am. J. syph. Gonor. and Ven. Dis.* 32: 301, 1948) showed that the factors were contained in the protein and not the lipid fraction of the yolk. Precipitation of the protein in alcohol-ether mixture and prolonged extraction with petroleum ether did not destroy the factor or remove it from the protein. After hydrolysis of the protein by acid or pancreatin it no longer supported growth. If the protein or egg yolk were autoclaved after addition to the medium, it still supported growth but the Donovan body did not produce capsular material. This suggests that the yolk protein may contain heat stable and heat labile factors. The factors could not be separated from the protein by dialysis. A large number of vitamins, amino acids and other 'growth substances' tried in combinations did not support growth when used in place of egg yolk in the medium. Similarly fresh liver, liver extracts, yeast, yeast extracts, milk, and beef serum also failed to support growth. (This investigation was supported by research grants from the Natl. Inst. of Health, Public Health Service.)

Fate of C^{14}-diethylstilbestrol in adult female rats. Donald J. Hanahan, Hyp J. Dauben, E. G. Daskalakis, T. Edwards and R. J. Meikle (introduced by Hans Neurath). *Dept. of Biochemistry, School of Medicine, and Dept. of Chemistry, Univ. of Washington, Seattle.*

Diethylstilbestrol, labeled with C^{14} in the methylene group, was administered subcutaneously in a dilute alkaline solution to adult female rats. At the end of a 6-hour period, various organs and tissues, expired CO_2, and excreta were analyzed for their C^{14} content. The results may be summarized as follows: *1*) no C^{14} was detected in the expired CO_2, and less than 2% of the injected activity was found in the urine; *2*) over 85% of the recoverable activity (50–75%) was found in the intestinal contents; and *3*) the remainder of the detectable activity was distributed in the brain, liver, and blood. Under the same conditions C^{14}-diethylstilbestrol was administered to bile fistula adult female rats. At the end of 6 hours the bile was analyzed for its C^{14} content. Seventy to 80% of the injected activity was found in the bile. The significance of these findings will be discussed.

Basis for experimental renal hypertension. Philip Handler and Frederick Bernheim. *Depts. of Biochemistry and Nutrition and of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.*

Subtotally nephrectomized rats develop hypertension when fed a diet containing sufficient protein. On low protein diets such rats remain normotensive. Administration of one mg. of ACTH rapidly restored the hypertension. Cortisone, but not desoxycorticosterone acetate, also restored the hypertension. Administration of ACTH was without effect on the blood pressure of the intact rat or unilaterally nephrectomized rat but did create hypertension in the bilaterally nephrectomized rat. Since this suggested that the excretory function of the kidney was involved, subtotally nephrectomized rats, fed a low protein diet, were used to assay urine for a 'pressor' compound. Normal human urine, diluted 1:1 with water was given intreperitoneally in one ml. doses. Within 48 hrs. systolic blood pressure rose 50 mm. and after 3 days of dosing, blood pressure remained significantly elevated for 2 weeks. A mock urine, reconstituted from known urinary constituents, was without effect. The active material was poorly dialyzable, not extracted by ethyl ether at pH 6, destroyed in 10 minutes at pH 1 and 100°, stable to pH 12 at 100° for 10 minutes, and not destroyed by crystalline trypsin.

Desoxyribonucleic acid content per nucleus in rat thymus damaged by β-radiation. Helen Harrington and Paul S. Lavik (introduced by J. R. Leonards). *Atomic Energy Med. Research Project, Western Reserve Univ., Cleveland, Ohio.*

We previously reported a marked decrease in the total amount, as well as in the concentration, of desoxyribonucleic acid (DNA) in thymus glands of adult rats given an LD_{50} dose of P^{32} (*Federation Proc.* 9: 194, 1950). One explanation for this effect, suggested by apparent degenerative changes seen upon histological examination of such tissues, is that the lymphocytes, supposedly highly sensitive to radiation, disintegrate *in situ* under the influence of the ionizing radiation. The cellular debris, including DNA or its fragments, is then presumably removed, leaving a tissue consisting essentially of stroma. However, when the nuclei and cytoplasmic fractions of rat thymus were prepared at intervals after the administration of an LD_{50} dose of P^{32}, the amount of DNA per nucleus did not differ from that found in simultaneously prepared nuclei from normal thymus glands. Degradation of DNA within structurally intact nuclei therefore appears unlikely. That the nuclei themselves disintegrate, liberating DNA into the cytoplasm, is also unlikely since the amount of DNA found in the cytoplasm was small and corresponded to the amount found in normal cytoplasm. It is concluded that a normal or an increased rate of removal of intact nuclei (probably lymphocytes) from the thymus gland, coupled with an impairment in the formation of new cells, probably accounts for the rapid loss of DNA and other tissue components from the thymus of rats receiving a toxic radiation dose.

Chemical studies on the ACTH protein and peptide mixture. J. IEUAN HARRIS* AND CHOH HAO LI. *Dept. of Biochemistry, Univ. of California, Berkeley.*

The data of Li *et al.* (*Arch. Biochem.* 9: 258) indicated that in the intact ACTH protein free amino groups are essential for hormone activity. The essentiality of free amino groups has been confirmed by the preparation of DNP and carbobenzoxy derivatives of the intact protein, and of a partially purified peptide mixture derived from it by peptic hydrolysis; these derivatives showed no hormone activity. The acylated protein derivatives were resistant to peptic digestion. Esterification of the protein and peptide mixture using a 0.1 N solution of HCl in methanol also resulted in total inactivation. This would seem to indicate the essentiality of free COOH groups; attempts to restore activity by saponification at pH 11 were unsuccessful, although control experiments revealed that the hormone preparations were not inactivated under the conditions of saponification. The purified protein hormone has a total sulphur content of 2.4%; in the peptide mixture total S falls to 1.3%. After shaking with Raney nickel for 6 hours at pH 9 the sulphur content of the active peptide fraction was further reduced to approximately 0.6%; partial desulphurisation did not lead to appreciable loss of hormone activity. A cystine content of 7.2% indicates the presence of 6–7 S-S linkages per protein molecule. The effect of reducing agents of both the protein and peptide mixture has been studied; when treated with a large excess of $SO_3^=$, mercaptoethanol and thioglycollic acid at pH 8, there was no loss of hormone activity. Potentiometric titration with Ag^+ at pH 10 by the method of Duggan, E. L. (unpublished) confirmed the absence of free SH groups in both the protein and peptide mixture; after sulphite treatment, silver titration revealed a partial cleavage of S-S bonds in the protein, and in the case of the peptide fraction it is probable that complete breakage of S-S bonds was achieved. Solutions thus treated were found to retain complete ACTH activity. These results suggest that S-S bonds are not essential for ACTH activity; it is hoped that experiments now in progress will conclusively establish the role of sulphur containing amino acids in ACTH.

Oxidation of acetaldehyde by glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle. JANE HARTING (introduced by SIDNEY F. VELICK). *Dept. of Biochemistry, Washington Univ. School of Med., St. Louis, Mo.*

The oxidation of glyceraldehyde is known to be catalyzed at about 1/1000th the rate of glyceraldehyde-3-phosphate by the crystalline rabbit muscle dehydrogenase. The mechanisms of the reactions appear to be the same. Acetaldehyde is also oxidized by the enzyme. Optical measurements based upon the appearance of reduced DPN indicate a rate about 1/100th that of free glyceraldehyde. The reaction likewise requires either phosphate or arsenate. The product with phosphate ion has the properties of acetyl phosphate. There is an increase in labile organic phosphate. A substance appears which reacts with hydroxyl amine to give the characteristic color of acetyl hydroxamic acid with ferric chloride. The hydroxamic acid derivative has the same chromatographic mobility as acetyl hydroxamic acid. Starting with acetyl phosphate and DPNH, the reaction goes in the reverse direction as determined by the disappearance of the absorption band of DPNH at 340mμ. Acetate ion itself does not affect the reversal. Although the physiological significance of the reaction is still problematical, the formation of acetyl phosphate by a mammalian enzyme is of interest.

Effect of insulin on oxygen uptake of rat adipose tissue in vitro. NIELS HAUGAARD,* JULIAN B. MARSH* AND WM. C. STADIE. *Dept. of Research Medicine, Univ. of Pennsylvania, Philadelphia.*

The respiration of strips and minces of retroperitoneal fat from the normal rat has been studied *in vitro*. This tissue was found to have considerable

metabolic activity and to oxidize a number of substrates studied. Insulin increased the oxygen uptake in the presence of glucose, succinate, lactate, pyruvate or acetate. Insulin had no effect in the absence of substrate, indicating that it in some way influences the metabolism of the substances studied. In the case of pyruvate, the increased oxygen uptake was associated with an increased substrate utilization. The oxygen uptake was essentially linear with time whether or not insulin was present, the rate with insulin being greater during the entire period of observation. The average increase in oxygen uptake with insulin was of the order of 25%. The experiments support the view that insulin directly or indirectly may influence oxidative reactions. Strips of adipose tissue exposed to insulin for a short time and washed exhibited an increased oxygen uptake when compared to controls. This is a demonstration that insulin combines with this tissue in a manner similar to its combination with the rat diaphragm. This finding strengthens the concept that a chemical combination of insulin with tissue is a pre-requisite for its physiological action.

Assay of biological activity of steroids by determination of renin-substrate (hypertensinogen). O. M. HELMER AND R. S. GRIFFITH.* *Lilly Lab. for Clin. Research, General Hosp., Indianapolis, Ind.*

Bilateral adrenalectomy of normal rats causes a reduction of the level of renin-substrate in the plasma (Gaudino, 1940). Treatment of these animals with DCA or Compound E restores renin-substrate to normal or near normal values. Renin-substrate was determined in adrenalectomized rats before and after the administration of steroids. Thirty-five steroids were assayed. DCA was the most active. Assigning the value of 100 to DCA, 'A' = 40, 'E' = 24, 21-acetoxypregnenolone = 4, 'S' = 1.5, and 'G' = 0. Compound E and DCA did not elevate the renin-substrate of normal rats whereas ACTH caused increased renin-substrate production in both normal and hypophysectomized rats.

Enzymatic formation of inorganic pyrophosphate from ATP. LEON A. HEPPEL AND R. J. HILMOE.* *Natl. Insts. of Health, Bethesda, Md.*

The mechanism of adenosine triphosphate (ATP) splitting has been investigated, with bull seminal plasma as a source of ATPase. Two distinct pathways were found, indicated by equations (1) and (2): (1) ATP→ adenosine-5′-phosphate + 2 orthophosphate. (2) ATP→ adenosine-5′-phosphate + inorganic pyrophosphate (PP). These reactions are the result of the presence of three different enzymes, which have been separated and partially purified. Two of them catalyzed reaction (1) while the other stimulated reaction (2). The ATPase which formed PP was relatively heat stable, had an alkaline pH optimum and did not appear to require any metal activator. Liberation of PP in stoichiometric amounts was demonstrated by analysis of the manganese salt and by quantitative assay with a specific yeast inorganic pyrophosphatase. A similar mechanism for ATP breakdown has been found in snake venom and bone (HITCHINGS, G. H. AND H. S. FULLER. *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.* 128: xlv, 1939; ZELLER, E. A. *Arch. Biochem.* 28: 138, 1950). The formation of PP from ATP may occur and be obscured by the presence of inorganic pyrophosphatase in the same enzyme preparation. In view of the widespread occurrence of inorganic pyrophosphatase, the true nature of ATP splitting in other tissues should be re-examined.

Adenosine triphosphate formation due to succinate oxidation. DAVID F. HERSEY AND SAMUEL J. AJL (introduced by M. D. KAMEN). *Dept. of Bacteriology and Immunology, Washington Univ. School of Medicine, St. Louis, Mo.*

Recent investigations (Hersey and Ajl, *J. Gen. Physiol.* 34: 295, 1951) have suggested that inorganic phosphate is esterified when succinate is incubated aerobically with cell-free extracts of *E. coli* in the presence of inorganic phosphate. The compound containing the acid-labile phosphate was not identified. By use of P^{32}, carrier adenosine triphosphate and paper chromatography, it has been possible to show that the compound containing the acid-labile phosphate has the same R_f as adenosine triphosphate. In addition, by determining the end products of succinic acid oxidation, it was found that the incorporation of P^{32} into carrier adenosine triphosphate is a result of the single oxidation step between succinate and fumarate (4%)-malate (96%) equilibrium.

A germination inhibitor from apple and celery seeds. SELMA S. HICKS,* D. M. DOTY* AND F. W. QUACKENBUSH. *Dept. of Agricultural Chemistry, Purdue Univ., Lafayette, Ind.*

A germination inhibitor was extracted from dormant apple and celery seeds and concentrated 50-fold by precipitation methods. The accumulated evidence indicated that the active substance was proteinaceous since it was precipitated by alcohol and by pH adjustment, was non-dialyzable, heat labile, and gave positive biuret and xanthoproteic tests. The most highly purified preparation of the celery seed extract contained nitrogen equivalent to 91.3% protein on a dry weight basis and its activity was 50-fold that of the crude extract. The most highly purified preparation from apple seed extract contained nitrogen equivalent

to 56.3% protein and its activity was 40-fold that of the crude extract. The inhibitory substance also acted as a competitive inhibitor for the proteolytic enzyme system of an extract from germinating sweet clover seeds. This *in vitro* activity may serve as a clue to its mode of action *in vivo*.

Products of galactose metabolism by *Brucella* species. K. HIGUCHI,* T. H. SANDERS* AND C. R. BREWER. *Camp Detrick, Frederick, Md.*

A culture of *Brucella suis* grew well in a complex medium containing 1% of either glucose or galactose. The glucose-grown cells upon sonic disintegration yielded an enzyme preparation which, aerobically, rapidly consumed galactose but not glucose. Oxygen consumption during galactose utilization indicated a partial oxidation. No detectable acid was produced, but a sugar-acid derivative was considered a possibility. Such a compound was indicated by the formation of an aldehyde-acid by periodic acid oxidation. The 2,4-dinitrophenylhydrazone of the probable aldehydo-acid was soluble in carbonate and its melting point corresponded to the same derivative of glyoxylic acid. Sugar determinations following acid hydrolysis of the enzymatic reaction mixture gave no evidence of polysaccharide formation. A culture of *Brucella melitensis* grew well in a complex medium containing 1% glucose, but only one-fifth as well with galactose. Changes of pH during incubation showed acid production in the galactose medium; this was not apparent in the glucose medium. After 48 hours incubation, a keto-acid amounting to as much as 1.0 mg/ml. (determined as sodium pyruvate) was found in the galactose culture. A smaller amount (0.2 mg/ml.) of the keto-acid was also found in the glucose culture. The pentoses, D-xylose and L-arabinose, whose configurations of carbons 1, 2 and 3 correspond respectively with those of D-glucose and D-galactose gave results which paralleled those obtained with the hexoses.

Enzymatic release of citrovorum factor. C. H. HILL AND M. L. SCOTT (introduced by L. C. NORRIS). *Cornell Univ., Ithaca, N. Y.*

An unidentified factor required by leuconostoc citrovorum appears to be chemically related to pteroylglutamic acid (PGA). Since much of the PGA occurring in nature is conjugated with glutamic acid, studies were undertaken to determine if the citrovorum factor (CF) also occurs in bound form and if CF can be released from its conjugate(s) by hog kidney enzyme preparations known to contain PGA conjugase. A sample of dried brewer's yeast, which was found to contain 1200 CF units per gram before incubation, was found to contain 15,300 CF units per gram after incubation overnight at 37°C. with the hog kidney enzyme preparation. This indicates the presence of a CF-liberating enzyme in hog kidney. Studies on the effect of cysteine and H_2S on the CF-liberating enzyme preparation have shown that this enzyme, like PGA conjugase, is activated by the addition of these substrates. Cysteine, as in the case of PGA conjugase, is a more effective activator on a molar basis than is H_2S. Sodium thioglycollate, which has been shown (Hill & Scott, in press) to inhibit competitively activation of PGA conjugase by cysteine was found to inhibit competitively the activation of the CF-liberating enzyme by cysteine. This is evidence that the enzyme responsible for the release of CF from its bound form contains an active sulfhydryl group which is transferred from cysteine by cysteine desulfhydrase. Since transulfhydration from cysteine also appears to be required for the activation of PGA conjugase, these results indicate a striking similarity between the CF-liberating enzyme and the PGA conjugase of hog kidney.

Utilization of oxalacetate and succinate in denervated muscle, unexercised and exercised by electrical stimulation. ROBERT M. HILL, DORSEY E. HOLTKAMP* AND ENID K. RUTLEDGE.* *Depts. of Biochemistry and Pathology, Univ. of Colorado School of Medicine, Denver.*

We have reported (*Federation Proc.* 9: 184, 1950) that there is a decrease of the oxalacetate utilization in muscle tissue adjacent to a methylcholanthrene-induced fibrosarcoma in the thigh of a rat as compared with corresponding muscle tissue of the opposite normal leg when O_2 uptake is used as a measure. Later, we found that this is similarly true of muscle tissue adjacent to glass-marble implants or to paraffin-injected sites, and of tissue taken from the quadriceps femoris muscle, the insertion of which had been severed. Succinate utilization also is decreased in all the above-mentioned conditions. We have now found that oxalacetate utilization is decreased to about one-third and succinate utilization to about two-thirds of normal in tissue of the unexercised quadriceps femoris muscle, the nerve supply of which was severed surgically 7–15 days previously. If, however, the denervated muscle was electrically stimulated to exercise without resistance (5-minute periods each at 8:00 A.M., 3:00 P.M. and 8:.00 P.M. daily), the oxalacetate utilization and the succinate utilization are each reduced only half as much as in the unexercised muscles. The stimulation was accomplished by using about 3.5 milliamperes of surging alternating current of 15 cycles per second as produced by a low-voltage variable-frequency generator.

Antimetabolite effects of substances which contain the 2,4-diaminopyrimidine moiety. GEORGE H. HITCHINGS, ELVIRA A. FALCO, PETER B. RUSSELL AND HENRY VANDERWERFF. *Wellcome Research Labs., Tuckahoe, N. Y.*

The growth of *Lactobacillus casei* in the presence of folic acid but in the absence of purines is inhibited by 2,4-diaminopyrimidines and condensed pyrimidine systems which contain this moiety. These substances may be arranged in a spectrum of inhibitors on the basis of reversal experiments with added folic acid or purines. Many 5- and 6-substituted pyrimidines lie at the anti-folic acid end of this spectrum while representatives of a limited number of condensed systems lie at the antipurine end. Antimicrobial activities of widely varied types are exhibited by 5-substituted diaminopyrimidines. To a considerable extent the nature of these activities is dependent on the chemical nature of the 5-substituent. Reversal studies, *in vitro* and *in vivo* suggest that these pyrimidines act as antimetabolites on a system closely related to but not identical with the folic acid system.

Effect of beryllium on plant phosphatase. MAHLON B. HOAGLAND (introduced by LEWIS L. ENGEL). *Med. Labs. of Collis P. Huntington Memorial Hosp. of Harvard Univ., Mass. General Hosp., Boston.*

The ability of beryllium to induce osteogenic sarcomas; its magnesium-competitive inhibitory action on animal alkaline phosphatases; and its growth-stimulating effect on magnesium-deficient plants (tomato and algae) suggested the present study of its effect on plant phosphatase. The enzyme preparation was the soluble cytoplasmic protein derived from the leaves of young tomato plants, precipitated by dialysis. Since the glycerophosphatase and ATPase activity of this preparation differed in susceptibility to heat, at least two enzymes were postulated, although competitive substrate inhibition studies indicated only one substrate could be split at a time. The effect of Be, Mg, Ca, Fe, Zn, Cu, Mn, and Al on the splitting of ATP and glycerophosphate was investigated. All but Mg and Ca inhibited the splitting of one or both substrates in varying degree. The action of Be was unique, however, in that it caused a more marked inhibition of ATP splitting by several hundred per cent (both absolutely and in terms of the ratio ATP split to glycerophosphate split). This phenomenon was manifest when the substrates were incubated separately or jointly with the enzyme preparation, and degree of inhibition depended (inversely) on substrate concentration. The inhibitions studied were reversible by dialysis. This ATP-sparing action of Be is of interest in view of the proliferative responses induced by Be in plant and animal tissues.

Value of supplementation of protein hydrolysates with the limiting essential amino acids in human nutrition. WILLIAM S. HOFFMAN AND GORDON C. MCNEIL.* *Hektoen Inst. for Med. Research, Cook County Hosp., and Univ. of Illinois College of Medicine, Chicago.*

Nitrogen balance experiments were performed in 7 normal male subjects to determine the nitrogen requirement for equilibrium (NE) when the only significant source of nitrogen was a lyophilized modified casein hydrolysate given orally. Then NE was determined for the same hydrolysate fortified with D,L-tryptophane, L-phenylalanine, and D,L-methionine in quantities that made the quantitative pattern equivalent to that found by Rose as minimal requirements of essential amino acids in humans. The mean NE with unsupplemented hydrolysate was 52.5 ± 3.5 mg/kg/day; for the supplemented product 38.5 ± 2.1 mg. The reduction of NE of 26% was statistically significant. At nitrogen equilibrium with the unsupplemented product, intake of L-tryptophane and methionine was equal to the minimal amounts found required by Rose. Therefore these amino acids were the limiting factor for NE for the product. However for the supplemented product nitrogen equilibrium was reached when all essential amino acids were still present in twice the quantity Rose found for minimal requirements. Therefore with an ideal mixture of essential amino acids, the limiting factor was the total quantity of nitrogen. This was corroborated in short experiments in which 15 gm. of added glycine changed from negative to positive the balance produced by a diet of 30 mg/kg/day of the supplemented product.

Products of desoxyribose nucleosides degradation by *Escherichia coli*. C. E. HOFFMANN AND L. A. MANSON (introduced by J. O. LAMPEN). *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Previous work with this organism (*Federation Proc.* 9: 397, 1950) has shown that washed, resting cells of *E. coli* (ATCC 9723) can degrade the desoxyribose from thymidine or hypoxanthine desoxyriboside. Evidence was obtained that the nucleosides underwent an initial phosphorolysis and the resulting desoxyribose phosphates were degraded by the organism. Further studies have been carried out to determine the products formed by anaerobically-grown cells in the fermentation of the desoxyribose from thymidine or hypoxanthine desoxyriboside. Under anaerobic conditions, 1.1 moles of acid and 0.25–0.35 moles of gas were formed per mole of desoxyribose disappearing.

With alkali in the center well of the Warburg cup, 0.8 moles of gas were produced. No keto acids, lactic acid or compounds reducing Cu^{++} were found at the end of the fermentation. The fatty acids produced were determined chromatographically. Alcohols were determined by oxidation to the corresponding acid and determining the latter chromatographically. Acetic acid, formic acid, ethanol and a trace of propionic acid were found. It appears as if the greatest part of the desoxyribose was fermented according to the following equation:

$$C_5H_{10}O_4 + H_2O \rightarrow CH_3COOH + CH_3CH_2OH + HCOOH(CO_2 + H_2)$$

A cell-free extract prepared by sonic vibration of anaerobically-grown cells was found to degrade hypoxanthine desoxyriboside to hypoxanthine and products not giving a desoxyribose test. This disappearance of desoxyribose was inhibited by small amounts of inorganic phosphate. Extracts from aerobically-grown cells were inactive. These results will be discussed.

Growth promotion by α-keto and α-hydroxy acids and its relation to vitamin B_6. JOSEPH T. HOLDEN,* RUTH WILDMAN* AND ESMOND E. SNELL. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

The ability of α-keto and α-hydroxy acids corresponding to valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan and histidine to replace these essential amino acids for *Streptococcus faecalis*, *Lactobacillus arabinosus*, and *Leuconostoc mesenteroides* was studied. In the complete medium, growth occurred both in the presence and absence of vitamin B_6. Under both conditions, single omission of any of these amino acids prevented growth. In most cases, the essential amino acid could be replaced by the corresponding keto or hydroxy acid, but only when vitamin B_6 was added. Since vitamin B_6 was not required for growth when the amino acid was supplied, this demonstrates a specific requirement for the vitamin for conversion of the keto or hydroxy acid to the corresponding amino acid by the intact organism. Individual variations in the activity of the various keto and hydroxy acids were noted. Dried cells of *S. faecalis* grown with vitamin B_6 converted the various keto acids to the corresponding amino acids when glutamic acid was present. This conversion was not carried out by cells grown without vitamin B_6, except when pyridoxal phosphate was also added to the cell suspension. This organism could utilize for growth only those hydroxy acids that were dehydrogenated by dried cells. Thus, in this organism, utilization of all keto acids in place of the essential amino acid appears to proceed only *via* transamination; and utilization of hydroxy acids appears to proceed *via* dehydrogenation to the keto acid and subsequent transamination.

Stability of cytochrome c in solution. W. L. HOLMES* AND A. M. WYNNE. *Dept. of Biochemistry, Univ. of Toronto, Ontario, Canada.*

The catalytic activities of 17 preparations of cytochrome c stored in solution for several months at 4°C. were determined at intervals by measuring oxygen-consumption in the ascorbate-reduced cytochrome–cytochrome-oxidase system. Though the cytochrome content of individual solutions, as indicated by spectrophotometric assay, showed no significant change over a period of several months the catalytic activity of the cytochrome, for a given unit of cytochrome calculated on the basis of the extinction coefficient, appeared gradually to diminish during this period at a rate corresponding to 0.2–0.4% each week. Because of the necessity of using a freshly prepared tissue-homogenate in each experiment and owing to variations in the cytochrome oxidase activity of different homogenates, changes in the catalytic activity of the added cytochrome were followed quantitatively in terms of the Michaelis constant. Preliminary analysis of kinetic data showed that under the experimental conditions adopted the system operates in zone A of Straus and Goldstein. During the storage of cytochrome c solutions prepared by the method of Keilin and Hartree a nitrogen-containing non-porphyrin fragment is detached from the cytochrome, and this fragment was shown to act as a competitive inhibitor of cytochrome oxidase in a system containing highly purified cytochrome c. When tested in the succinoxidase system preparations of cytochrome c, shown previously in the ascorbate-reduced system to differ in catalytic activity though present in equivalent concentrations calculated from spectrophotometric data, were found to have equivalent activities when present in equivalent concentrations.

Ketopentose-phosphate formation in the enzymatic degradation of phosphogluconate. B. L. HORECKER AND P. Z. SMYRNIOTIS.* *Natl. Insts. of Health, Bethesda, Md.*

An enzyme has been purified from yeast which catalyzes the oxidation of 6-phosphogluconate by triphosphopyridine nucleotide (TPN) (*Federation Proc.* 9: 185, 1950). The enzyme has a high affinity for phosphogluconate and for TPN, with dissociation constants of 5×10^{-5} and 1×10^{-5} moles per liter, respectively. The ultimate product of the oxidation of phosphogluconate with the purified yeast enzyme is ribose-5-phosphate which is formed from a ketopentose-phosphate intermediate. (*Arch. Biochem.* 29: 232, 1950). This precursor,

purified from the reaction mixture by means of ion-exchange chromatography, has the following properties: The specific rotation based on the orcinol pentose assay is $[\alpha]_D^{20°} = -40°$. It is split by acid much more rapidly than is ribose-5-phosphate and is resistant to oxidation by Br_2. After removal of the phosphate by a phosphatase from potato, it yields a two-banded spectrum in the orcinol reaction which is identical with that given by the ketopentose ribulose. These properties, as well as the enzymatic conversion to ribose-5-phosphate, suggest that the intermediate is ribulose-5-phosphate. The oxidation of 6-phosphogluconate in the 3-position followed by decarboxylation would result in the formation of ribulose-5-phosphate.

Determination of dehydroisoandrosterone. BENJAMIN N. HORWITT (introduced by HERBERT P. SARETT). *Dept. of Medicine, Tulane Univ., Alton Ochsner Med. Fndn., New Orleans, La.*

The reaction of sulfuric acid with dehydroisoandrosterone (DHA) has been applied to the determination of DHA in pure solutions and urinary extracts. Conditions have been determined for a rapid, selective, and reproducible spectrophotometric determination of DHA that is simple and more sensitive than those previously reported. (JENSEN. *Acta Endocrinol.* 4: 140, 1950; HANSEN. *Endocrinol.* 46: 207, 1950). Unlike the above methods, the presence of androsterone up to at least eight times the amount of DHA does not interfere with the determination of DHA. To a dried aliquot, 4 ml. of a 95% alcohol-sulfuric acid mixture (1:4) are added and placed in a boiling water bath for one minute. Cool and add 6 ml. of 95% alcohol. Cool and read at 590 mμ. The optical density $\times 10^4$ per γ varies from 101 at 20γ to 105 at 80γ. Of 40 steroids of the C-19 or C-21 types examined, only DHA and its acetate gave a blue-violet color which had an adsorption peak only at 590 mμ. When applied directly to urinary extracts prepared for the determination of 17 ketosteroids, values for DHA were obtained which varied from 7–30% of the total ketosteroids. Recoveries of DHA when added to urinary extracts were in the order of 85%.

Use of serum albumin as source of serum factor in pneumococcal transformation. ROLLIN D. HOTCHKISS AND HARRIETT EPHRUSSI-TAYLOR.* *Hosp. of Rockefeller Inst., New York City.*

In order to demonstrate the transformation of pneumococcal types, suitable pneumococci of a competent R (rough) strain are inoculated into a medium containing, besides constituents adequate for maintaining growth, anti-R agglutinins and the specific nucleic acid transforming factor. McCarty, Taylor and Avery (*Cold Spring Harbor Symp., Quant. Biol.* 11: 177, 1946) showed that an additional factor or factors provided by serum or serous fluids was necessary before transformation was successfully produced. It has now been demonstrated that the last factor can be adequately supplied by suitable bovine and human serum albumin preparations. That serum albumin itself possesses this activity is supported by the following findings: *a*) albumin preparations separated from bovine or human plasma by salt or alcohol fractionation are highly effective in the transforming system, *b*) several times recrystallized albumins are equally effective, as is *c*), albumin from human serum highly purified through its mercury derivative, *d*) denaturation by heat destroys the activity of bovine albumin and the activity is restored by acid treatment simultaneously with a restoration of the solubility in salt typical of albumin, and *e*) the activity of albumin preparations is prevented by the addition of certain crude serum globulin preparations. The last observation probably explains why many whole sera are inactive or not as active as the human pathological chest or abdominal fluids previously used as the source of this factor in transformation systems.

Mechanism of rhodopsin synthesis. RUTH HUBBARD (introduced by GEORGE WALD). *Biological Labs. of Harvard Univ., Cambridge, Mass.*

We have suggested earlier that rhodopsin is synthesized via the oxidation of vitamin A_1 to retinene$_1$ by cozymase and retinene reductase, coupled with the spontaneous condensation of retinene$_1$ with rhodopsin-protein (opsin) to form rhodopsin (WALD AND BROWN, WALD AND HUBBARD. *Proc. Nat. Acad. Sc.* 36: 84, 92; 1950). It has now been shown that the addition to retinal homogenates of factors which promote the oxidation of vitamin A_1, aids in all cases the synthesis of rhodopsin. Such factors include vitamin A_1 itself, cozymase, and oxidative systems such as heart particle 'succinoxidase' which keep cozymase oxidized. Homogenates of the pigment epithelium of the eye promote rhodopsin synthesis by supplying the retinal tissue with vitamin A_1. The pigment layers of the eye contain also a heat-labile, water-soluble factor which stimulates rhodopsin synthesis from vitamin A_1. Through its dependance upon cozymase, the rhodopsin system is linked with retinal fermentation and respiration, and these in turn depend for oxygen and metabolites upon exchanges with the blood circulation of the choroid by diffusion through the intervening pigment epithelium. The crystalline horse liver alcohol dehydrogenase of Bonnichsen catalyses the equilibrium between vitamin A_1 and retinene$_1$, and so can substitute for retinene reductase. A system has been assembled from four

components in water solution, which synthesizes rhodopsin and performs all the main reactions of the rhodopsin system. This contains opsin, vitamin A_1, cozymase, and liver alcohol dehydrogenase. The yield of rhodopsin obtained in this system depends markedly upon the stereoisomeric form of vitamin A_1 that is employed. (This research was supported in part by the Medical Sciences Div. of the Office of Naval Research.)

A modified Knoop test for histidine. ANDREW HUNTER. *Dept. of Pediatrics, Univ. of Toronto, and Hosp. for Sick Children, Toronto, Ontario, Canada.*

The following modification of the Knoop test for histidine is at least as sensitive as any hitherto proposed, and has the unique advantage that the distinctive colour is developed without application of heat. To 5 ml. of a nearly neutral unbuffered solution of histidine, containing not more than 0.35 mg. of the base, add 0.5 ml. of 2N H_2SO_4 (giving a pH of about 1.0) followed, under a dim light, by 0.25 ml. of a neutral bromine solution (0.5 ml. dissolved in 50 ml. of tertiary butyl alcohol and diluted with an equal volume of water). Set the mixture in complete darkness for from 1-3 (optimally 2½) hours. Add then, to destroy residual bromine, 6 drops of a 3% solution of As_2O_3 in 1% NaOH. Add lastly 1 ml. of a solution prepared by dissolving 100 gm. of sodium acetate crystals in 100 ml. of water containing 3.1 mg. of $CuSo_4$, 5 H_2O. In the mixture, which should have a pH of about 5.17, there will presently develop a characteristically brilliant rose-violet colour (maximum transmission at 540 mμ) which reaches its greatest depth in 15 minutes. In the absence of the prescribed trace of copper the colour obtained is a dull purple of greatly inferior intensity. In pure histidine solutions the test is unmistakably positive with as little as 0.025 mg. of the base (1 part in 200,000). The colour is somewhat enhanced by low concentrations of amino-acids, and greatly diminished by urea. The utilization of the test for quantitative determination of histidine is now under study.

Effect of dinitrophenol on phosphorylations coupled with oxidation of α-ketoglutarate. F. EDMUND HUNTER, JR. AND SIDNEY SPECTOR.* *Pharmacology Dept., Washington Univ., Saint Louis, Mo.*

Phosphorylation of glucose via hexokinase and ATP is rapid during oxidation of succinate, β-hydroxybutyrate, and α-ketoglutarate by washed liver particles. When increasing concentrations of dinitrophenol were tested for their 'uncoupling' effect on these phosphorylations, 5×10^{-5} M usually caused complete elimination of the phosphorylations due to succinate and β-hydroxybutyrate oxidation, but only partial elimination of phosphorylation coupled with α-ketoglutarate oxidation. With α-ketoglutarate the phosphorylation falls sharply up to 10^{-4} M dinitrophenol. Between this concentration and 10^{-3} M there is a gradual decline, but even at 10^{-3} M there is a residual phosphorylation resistant to dinitrophenol. The phosphorylation due to anaerobic oxidation of α-ketoglutarate with either iminoglutarate (α-ketoglutarate + NH_3) or oxalacetate as electron acceptor was very resistant to dinitrophenol. This phosphorylation, presumably at the substrate level, was unaffected by dinitrophenol concentrations causing complete elimination of phosphorylations due to succinate and β-hydroxybutyrate oxidation. Dinitrophenol at 4×10^{-4} M, which eliminates 75–80% of the phosphorylation due to oxidation of α-ketoglutarate with oxygen as electron acceptor, causes only 10–15% inhibition of phosphorylation when iminoglutarate or oxalacetate is the electron acceptor. These data suggest that the mechanism for creating the energy rich phosphate bond formed at substrate level in α-ketoglutarate oxidation is very resistant to dinitrophenol and perhaps basically different from the mechanisms higher in the electron transport chain to oxygen. The sensitivity of the latter mechanisms to dinitrophenol is illustrated by the cases of succinate and β-hydroxybutyrate. (Supported in part by a USPHS (NIH) grant.)

Chromatographic separation of phosphatases of snake venom. R. O. HURST* AND G. C. BUTLER. *Dept. of Biochemistry, Univ. of Toronto, Ontario, Canada.*

When the lyophilized venom of either Russell's viper or the rattlesnake (*Crotalus adamanteus*) was dissolved in water and passed through a column of cellulose most of the phosphodiesterase and all of the phosphomonoesterase were adsorbed and much inactive material passed into the filtrate. The diesterase was removed from the column by elution with 0.1% NaCl and the monoesterase was eluted subsequently with 1.0% NaCl. This procedure, and modifications of it, failed to achieve any separation of the phosphodiesterase and phosphomonoesterase activities of intestinal phosphatases prepared from dog faeces or from calves' duodena. The phosphomonoesterase from Russell's viper venom does not hydrolyse phenyl phosphate, α-glycerophosphate nor adenosine-3-phosphate. It does, however, hydrolyse adenosine-5-phosphate and the mononucleotides from thymus desoxyribonucleic acid. This provides indirect evidence that the phosphate ester may be at carbon 5 of the desoxyribose in these mononucleotides.

Effect of fasting upon lipogenesis in the intact animal. T. T. HUTCHENS, J. T. VAN BRUGGEN, C. K. CLAYCOMB AND W. J. CATHEY. (introduced by E. S. WEST). *Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.*

A tracer amount of C_{14}, carboxyl labeled acetate was given to 200-gram rats and CO_2 collected for one hour. The entire animal was then digested in alcoholic KOH and separated into saponifiable and non-saponifiable fractions. Four animals were used after one hour, fasting, 4 at 24 hours and then 2 each at 48, 72, 96 and 120 hours fasting. One hour after the last meal (one hour fast) the 200-gram rats were able to incorporate 1.0% of the administered acetate C_{14} into total body cholesterol, 4.0% into body fatty acids, and put out 47% of the activity in CO_2. Evaluating the remainder of the data on the basis of 100% at one hour fast time, the degree of incorporation of acetate into cholesterol at 24, 48, 96 and 120 hours, amounted to 83, 60, 31, 34 and 41% respectively. Fatty acids at these same intervals showed 53, 51, 39, 39 and 30% incorporation. The CO_2 collected, evaluated on the same basis showed 115, 126, 65, 71 and 86%. Although the rats after the 120-hour fast had a weight loss up to 50% and a total fatty acid loss of 80%, fatty acids isolated had specific activities similar to those isolated from the 24-hour fasted animals. Total cholesterol did not change after 120 hours fasting but cholesterol specific activities of 120-hour animals were lower than 24-hour animals. Dilution of labeled acetate by the excess breakdown of body lipid may account for some of the lowered total incorporation of acetate after prolonged fasting.

Interaction of quinoline and acridine derivatives with plasma proteins. J. LOGAN IRVIN, ELINOR MOORE IRVIN* AND FRANK S. PARKER.* *Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Md., and Dept. of Biological Chemistry and Nutrition, Univ. of North Carolina, Chapel Hill.*

In the physiological range of pH the 4-aminoquinoline, chloroquin, and the 9-aminoacridines, quinacrine and 2-methoxy-6-chloro-9-(1′-methyl-8′-diethylamino-octylamino)-acridine (SN-12868), exist as equilibrium mixtures of the two species B-B′H$^+$ and $^+$HB-B′H$^+$ in which B represents the ring-nitrogen and B′ the diethylamino nitrogen of the side-chain. Albumin and the proteins of fraction III-1 of bovine plasma interact strongly and reversibly with species B-B′H$^+$ but not with $^+$HB-B′H$^+$. The proteins of fraction II interact very weakly with B-B′H$^+$. These interactions are evaluated independently by spectrophotometric and by equilibrium-dialysis methods which will be described. The first association constant for SN-12868 versus bovine albumin is 4.4 times that for chloroquin at high ionic strength. At low ionic strength there is some deviation from the simple relationship for reaction with B-B′H$^+$ which can be accounted for fairly satisfactorily by means of a formulation which assumes electrostatic effects from groups adjacent to the 'binding site' of the protein. Other theoretical relationships have been derived which have a bearing upon the general topic of the shifting of equilibria between conjugate pairs of molecules or ions by preferential interaction of a protein with one member of the pair. These formulations, which are related to equations derived by W. M. Clark, can be applied in the systematic treatment of the protein error of indicators and in the physico chemical description of coenzyme-protein interactions.

Electrophoretic analyses of rat blood serum proteins and urinary proteins after injection of materials proposed as blood substitutes. ELOISE JAMESON (introduced by WALTER MARX). *Dept. of Medicine, Univ. of Southern California, Los Angeles.*

The experiments described here were carried out as part of a war time O.S.R.D., C.M.R. project under the direction of Dr. Thomas Addis of the Stanford Medical School. Electrophoretic analyses of rat serum and urinary proteins were made on material obtained from experiments in which the effects were observed, of repeated intraperitoneal injections (one, three and five times) of material proposed as blood substitutes. Oxy-poly-gelatins were from the California Institute of Technology and blood serum proteins from the Institute of Physical Chemistry, Harvard Medical School. Oxy-poly-gelatins separated by alcohol precipitation into particles of different sizes were used as criteria of the effect of particle size. The influence of the injection of increasing amounts of proteins (200 mg., 400 mg. and 800 mg. per rat) in one injection were also studied. In order to identify the proteins which had been injected, electrophoretic experiments were made on the material alone and after its addition to normal rat serum. Serum was diluted 1–3 or 4 of Cohn's pH 7.7 phosphate buffer. Urine was run undiluted. Some proteins as Beef globulins I, II, III-1 and ether-washed IV are largely retained in the serum while at the same time the normal serum proteins are very little changed. Those excreted rapidly and apparently unchanged as Bence Jones protein, hemoglobin, egg albumin and the smaller oxy-poly-gels, leave serum proteins very little changed from normal. When there is retention of proteins in the serum for a time, as for instance with some of the oxy-poly gelatins, beef albumin or beef globulin IV, a dilution of serum proteins occurs. In one of these cases the serum was returned to practically normal after 10 days.

Reaction of α-chymotrypsin with analogues of diisopropyl fluorophosphate (DFP). EUGENE F. JANSEN, A. LAURENCE CURL* AND A. K. BALLS. *Enzyme Research Div., Bureau of Agricultural and Industrial Chemistry, U. S. Dept. of Agriculture, Albany, Calif.*

Previous communications from this laboratory on the study of the mode of inhibition of α-chymotrypsin by DFP have demonstrated that the crystalline inert protein resulting as the inhibition product contains the isopropylphosphate residue in the proportion of one mole per mole of protein. The fluorine is eliminated from the DFP as HF. Hence the DFP reacts with the enzyme by a condensation reaction. Fleisher *et al.* (*Federation Proc.* 9: 171, 1950) found that the reaction of chymotrypsin with tetraethyl pyrophosphate was equally as quantitative as that with DFP; i.e., two ethoxy and one phosphorus per mole of protein were found in the crystallizable, inert protein resulting from this inhibition reaction. Hartley and Kilby (*Nature* 166: 784, 1950) arrived at similar conclusions regarding the inhibition of this enzyme by diethyl *p*-nitrophenyl phosphate. They found that the liberation of nitrophenol paralleled the amount of inhibition when chymotrypsin was treated with this analogue of DFP. We have now analyzed the crystalline, inert proteins resulting from the inhibition of α-chymotrypsin by each of five analogues of DFP (diphenyl chlorophosphate, diethyl thionofluorophosphate, tetraisopropyl pyrophosphate, diethyl *p*-nitrophenylphosphate, and tetrapropyl dithionopyrophosphate). In each instance one mole of phosphorus was introduced per mole of enzyme even when relatively large amounts of the analogue were needed to cause complete inhibition. Hence the inhibition reaction of α-chymotrypsin with DFP and its various analogues was found to be a general reaction.

Metabolism of radioactive nicotinic acid and nicotinamide in the rat. B. CONNOR JOHNSON AND PEI-HSING LIN.* *Div. of Animal Nutrition, Univ. of Illinois, Urbana.*

Nicotinic acid and nicotinamide labeled with radiocarbon in the carboxyl position were given by intraperitoneal injection to individual rats, and expired CO_2, urine and feces were collected. Radioautographs were prepared of paper-strip chromatograms of the urine from each rat. Following nicotinic acid administration the radioautographs showed the presence of 7 metabolic excretion products, of which 4 could be identified by their Rf values compared to known compounds. The approximate relative amounts of each product excreted are as follows: N^1-methylnicotinamide 2.7%, nicotinuric acid 60.9%, nicotinic acid 24.4%, nicotinamide 0.26, and three unidentified compounds which accounted for 3.5, 7.7 and 0.48%, respectively of the activity excreted. Following nicotinamide administration, 9 radioactive excretion products were found on paper chromatograms, of which 4 could be identified. The approximate relative amounts of each compound excreted were as follows: N^1-methylnicotinamide 66.9%, nicotinuric acid 0.9%, nicotinic acid 6.6%, nicotinamide 4.1%, and 5 unidentified compounds which accounted for 6.1, 10.8, 0.8, 1.0 and 2.4%, respectively, of the activity excreted.

Seasonal variation in toxicity of Demerol in young and adult rats. WALTER H. JOHNSON* AND EMMETT B. CARMICHAEL. *Biochemistry Dept., Alabama Med. Center, Birmingham.*

Both young and adult healthy stock rats have been used in toxicity studies with Demerol hydrochloride. Two arbitrary weight-groups were selected: *a*) 100–199 gm. and *b*), 200–483 gm. A fresh aqueous solution was injected intraperitoneally. The rats received only a single dose each. The doses varied by 2.5 mg. increments from 60–85 mg/kg. for 94 rats used during the summer months, while the doses varied by 5-mg. increments from 65–95 mg/kg. for 123 rats used during the winter months. The smallest dose to cause death was 62.5 mg/kg. during the summer and 70 mg/kg. during the winter months. The survival time for those rats that died varied from 7–56 minutes, with the majority of them dying within 40 minutes. The LD_{50} of Demerol hydrochloride for the weight-group (100–199 gm.) during the summer and winter months seems to be in the range 72.5–80 mg/kg. for the former and 80–95 for the latter. The LD_{50} for the weight-group (200–483 gm.) being in the range 67.5–75 mg/kg. for the summer months and 75–85 mg/kg. for the winter months.

Excretion of vitamin B_{12} by human subjects. DOROTHY R. JUTTON* AND HELEN T. PARSONS. *Dept. of Home Economics, Univ. of Wisconsin, Madison.*

Intramuscular injection of 450 mg. vitamin B_{12} for 4 subjects (women) led to a urinary return of 37–50% of the dose within 6 hours and 40–75% within 12 hours, in agreement with reports of others on men. In agreement also was the almost complete failure to find vitamin B_{12} activity in the urine on normal diets or after oral dosage. After parenteral administration, a small amount of microbiological activity for *Lactobacillus leichmannii 4797* (A.T.C.C.) was detected in the samples of bile-rich duodenal contents within 30 minutes, while the basal specimens showed none. Observations on the vitamin B_{12} activity of the feces of these subjects gave no clear evidence of increases following dosage.

Mechanism of potassium activation of phosphate transfer. J. F. KACHMAR* AND P. D. BOYER. *Div. of Agricultural Biochemistry, Univ. of Minnesota, St. Paul.*

General equations have been developed which allow kinetic analysis of the mechanisms of enzyme activation. Assuming equilibrium analysis is valid, four cases considered are *1*) compulsory combination of the enzyme with the activator before combination with the substrate, *2*) compulsory combination of the enzyme with the substrate before combination with the activator, *3*) independent combination of the enzyme with either the substrate or activator, and *4*) compulsory combination of the activator with the substrate before formation of the enzyme-substrate complex. The equations developed have been applied to a study of the activation by potassium of the phosphoferase catalyzing the transfer of phosphate from 2-phosphopyruvate to adenosinediphosphate using a partially purified rabbit muscle enzyme system. The reaction velocities were determined by measurement of the pyruvate produced using the reaction of pyruvate with 2,4-dinitrophenylhydrazine. The results show that the activation by potassium corresponds to *alternative* 3 given above. The dissociation constant for the phosphopyruvate-enzyme combination is 9×10^{-5} and for the potassium-enzyme combination is 0.011. NH_4^+ and Rb^+, cations with ionic radii approximately the same as that of the potassium ion, also serve as activators. It has also been shown unequivocally that potassium is essential for the phosphate transfer, and is not merely an accelerator of the transfer as previously considered.

Enzymatic formation of oxalacetate from pyruvate and CO_2. J. P. KALTENBACH* AND GEORGE KALNITSKY. *Dept. of Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City.*

In the presence of pyruvate and high concentrations of bicarbonate (0.19 M) as substrates, the anaerobic formation of relatively large amounts of oxalacetate (approximately 200 μl.) by enzyme extracts of *Escherichia coli* or *Proteus morganii* can be demonstrated. Malonate did not inhibit the formation of oxalacetate from pyruvate and bicarbonate, nor was any oxalacetate formed from succinate, anaerobically, indicating that the oxalacetate did not arise via the tricarboxylic acid cycle. No acetoacetate was formed under these experimental conditions. The oxalacetic acid was identified by *1*) decarboxylation with aniline-citrate, $CuSO_4$ and acid heat, *2*) partition paper chromatography, *3*) chromatography and absorption spectrum of the 2,4-dinitrophenylhydrazone according to the method of LePage, and *4*) fixation of C^{14} in the β-COOH of the oxalacetic acid. No significant amounts of C^{14} were found in the α-COOH of oxalacetic nor in the COOH of pyruvic, acetoacetic or α-amino acids. Malic acid does not appear to be an intermediate in this reaction, since no C^{14} could be detected in this compound added as a carrier. The effects of various factors (such as extraction temperature, dialysis, aging, freezing, heating and inhibitors) are described on the formation of oxalacetate from pyruvate and bicarbonate as substrates. The optimum conditions for this carboxylation reaction, in addition to the enzyme, are *1*) pyruvate, 0.07–0.08 M; *2*) bicarbonate, 0.19 M; *3*) pH, 8.1; *4*) inorganic phosphate, 0.09 M; *5*) Mg^{++}, 0.07 M; and *6*) biotin, 20 μg. per flask or approximately 3.9×10^{-5} M.

Direct enzymatic deamination of adenine derivatives by takadiastase deaminase. NATHAN O. KAPLAN, SIDNEY P. COLOWICK AND FRANCIS E. STOLZENBACH.* *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

The deaminase from takadiastase (MITCHELL AND MCELROY, *Arch. Biochem.* 10: 351, 1946) has been found to deaminate directly DPN; the rate of deamination of the coenzyme being approximately one-tenth of that of adenosine. Desamino DPN has been isolated from the enzymatic reaction mixture and found to be identical with the chemically prepared compound in physical properties and in its diminished activity towards yeast alcohol dehydrogenase and *Neurospora* DPNase. Reduced DPN is also deaminated by the enzyme. TPN is not attacked by the enzyme. Treatment of TPN with prostatic phosphatase yields DPN, which is then deaminated by the takadiastase enzyme. 5-adenylic acid and adenylic acid 'b' are also directly deaminated; however, the amino group of adenylic acid 'a' is resistant to the action of the enzyme. The fact that the enzyme does not deaminate either TPN or adenylic acid 'a' confirms the finding of Kornberg and Pricer (*J. Biol. Chem.* 186: 557, 1950) on the presence of adenylic acid 'a' in TPN. The takadiastase enzyme also deaminates ATP but does not act on adenine, guanosine or guanylic acid. The affinities of the various adenine derivatives for the deaminase have been determined, and details of the further purification of the enzymes will be presented.

Growth inhibition by cortisone and related steroids. DAVID A. KARNOFSKY, LOIS P. RIDGWAY AND C. CHESTER STOCK (introduced by K. DOBRINER). *Sloan-Kettering Inst. for Cancer Research, New York City.*

Cortisone produces inhibition of growth, and characteristic modifications of development of the chick embryo, newly-hatched chick and new-

born mouse. These effects were used in studying more than 80 steroids for cortisone-like activity. In the chick embryo, Kendall's Comp. F Ac. had a cortisone-like effect at 0.015 mg. per egg, cortisone AC., 21-desoxycorticosterone AC. and Compound A Ac. were active at 0.25 to 0.45 mg. per egg. DCA at 0.9 mg. per egg, and for other steroids doses up to 9 mg. per egg were necessary to elicit cortisone-like activity. Cortisone activity appeared to be dependent on the presence of a 21 carbon steroid, a ketone group in the 20 position, a ketone or hydroxy group in the 3 position, and a Δ^4 or Δ^5 unsaturation. Saturated steroids of the allopregnane series have exhibited activity, whereas pregnane derivatives have been uniformly negative. Activity is enhanced by an 11 ketone group, and more so by an 11 B hydroxy group. In newly-hatched chicks, as in chick embryos, Comp. F Ac. was almost 100 times more active than cortisone Ac., and corticosterone was almost as active as Comp. F Ac. Cortisone and Comp. F Ac., in contrast to their differential effects in the chick, showed almost identical activity in new-born mice; at larger doses Comp. A Ac., 21-desoxycorticosterone Ac. and DCA also had a cortisone-like effect. The growth-inhibiting effects of steroids in relation to structure and dosage were compared in new-born mice, newly-hatched chicks and chick embryos.

Formation of CO_2, lipid and carbohydrate from C^{14}-labeled glycerol. M. L. KARNOVSKY AND L. I. GIDEZ (introduced by E. G. BALL). *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Glycerol, labeled with C^{14} was synthesized as follows: acetic acid → bromoacetic acid → cyanoacetic acid → calcium malonate → ethyl malonate → ethyl acetoxymalonate → glycerol. The final step was carried out with lithium aluminum hydride. The use of carboxyl-labeled acetate, or $NaC^{14}N$ to give cyanoacetic acid, yielded α-labeled glycerol; methyl-labeled acetate yielded β-labeled glycerol. When free α-labeled glycerol was administered to fasted rats, the expired CO_2 reached peak specific activity at 65–80 minutes. In 6 hours 35–50% of the administered counts were expired. The animal was sacrificed at the end of this period, and the tissue distribution of activity established. Liver lipids accounted for approximately 5% of the administered counts, most of the activity being in the glycerol moiety. The phosphatide fraction was about 1.5 times as active as the neutral triglyceride. All other fats together made up about 3% of the administered counts. The activity of the liver glycogen, which often approached that of the blood glucose, indicated that about 16% was derived from glycerol. Experiments with glycerol-labeled tributyrin and triolein, administered orally, showed that the first was rapidly split and the final results approximated those with free glycerol. Triolein was split slowly, retention of activity was very high, and the liver lipids, especially the phosphatides, were of significantly lower activity than in experiments with free glycerol.

Further purification and properties of 'malic' enzyme from *L. arabinosus*. SEYMOUR KAUFMAN (introduced by S. RATNER). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

Further purification of the 'malic' enzyme of *L. arabinosus* (*J. Biol. Chem.* 187: 891, 1950) has been effected by a procedure involving heat denaturation of other proteins in presence of Mn^{++} and malate, ammonium sulfate fractionation, protamine precipitation of nucleic acid, $Ca_3(PO_4)_2$ adsorption, and refractionation with ammonium sulfate in presence of Mn^{++} and malate. An 80-fold purification was achieved over the initial extract. Previous preparations of the enzyme catalyzed the dissimilation of *l*-malate to CO_2 and D,L-lactate. With the present preparations the product is L-lactate. It has been shown that D-lactate can be racemized by cruder preparations and that DPN, but not Mn^{++}, is required. This suggests that the racemization is due to the action of two lactic dehydrogenases with opposite optical specificities, one of which has been removed on purification. Lactic dehydrogenase has not been completely removed so far and it has not been possible to demonstrate an accumulation of pyruvate from *l*-malate. This aspect of the problem has been investigated with C^{14}. When *l*-malate, labeled with C^{14} in both carboxyl groups, is decarboxylated by *L. arabinosus* enzyme in presence of carrier lactate and pyruvate, the specific radioactivity of the lactate is much greater than that of pyruvate. This suggests that the pyruvate formed by oxidative decarboxylation of malate is reduced to lactate by lactic dehydrogenase more rapidly than it equilibrates with the pyruvate pool in the solution.

Enzymatic phosphorylation of riboflavin. E. B. KEARNEY AND SASHA ENGLARD (introduced by THOMAS P. SINGER). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

An enzyme catalyzing the reaction: riboflavin + ATP or ADP → riboflavin-5-PO_4 + ADP or AA has been isolated from an autolysate of Brewer's yeast, and the name 'flavokinase' is proposed. Previous claims (*Enzymologia* 6: 333, 1939) of extensive phosphorylation of riboflavin by inorganic phosphate, without the participation of ATP, in preparations of intestinal mucosa could

not be confirmed. ATP is an obligatory phosphorylating agent in view of the energetics of the reaction. The enzyme from yeast converts riboflavin stoichiometrically to riboflavin-5-PO_4 (FMN), but in crude preparations, the FMN is further converted to FAD by the enzyme described by Schrecker and Kornberg (*J. Biol. Chem.* 182: 795, 1950). The latter enzyme is removed on purification of 'flavokinase' by ammonium sulfate fractionation, dialysis, and ethanol fractionation. By this procedure a stable lyophilized powder is obtained which is 400–500-fold purified. 'Flavokinase' requires Mg^{++} for full activity; Mn^{++} and Co^{++} are active to a lesser degree, and Ca^{++} inhibits the enzyme competitively. The dissociation constants of the enzyme for ATP, riboflavin, and Mg^{++} have been determined. ATP and ADP seem to have identical K_M values (1.7×10^{-5} *M*), but the former is more effective as a phosphate donor. Inosine triphosphate is inactive. The reaction follows zero order kinetics, with a pH optimum of about 8.0 and a temperature optimum of 38°C.

Turnover of proteins of cell fractions of adult rat liver *in vivo*. ELIZABETH B. KELLER (introduced by PAUL C. ZAMECNIK). *Med. Labs. of Collis P. Huntington Memorial Hosp. of Harvard Univ. at Massachusetts General Hosp., Boston, Mass.*

DL-Leucine-1-C^{14} (0.1 mc/mM, synthesized by R. B. Loftfield) was given intravenously to 1–2 year old male Wistar rats (3.3 mg/100 gm). Liver samples were removed at different times from 15 minutes to 2 days, blendorized, and fractionated. Sedimentation of a nuclear fraction and of mitochondria (Mit) was from 0.88 M sucrose, of large microsomes (Mic-l) and intermediate microsomes (Mic-i) from 0.44 M sucrose. The remaining microsomes were precipitated at pH 5. The supernatant (S) from this precipitation was nearly free of nucleoprotein. Protein, C^{14} in protein, ribonucleic acid (RNA), and desoxyribonucleic acid (DNA) were determined. Recovery of each in the fractions was 90% or over. From the C^{14} in protein after 15 minutes, the protein leucine replaced in percentage per day was calculated. Analyses on the more homogeneous fractions are given.

	CENTRIFUGATION		LEUCINE REPLACED	RNA	DNA
	g	*min.*	*%/day*	*mg/gm protein*	*mg/gm protein*
Liver			19	32	7.9
Mit	12,000	10	5	12	0.4
Mic-l	30,000	20	30	63	19.5
Mic-l	40,000	60	42	64	13.4
S			14	2	0.4

At 15 minutes the microsome fractions contained 77% of the radioactive protein recovered. These data emphasize the importance of the microsomes in the process of incorporation of amino acids into protein in the liver.

Effect of *in vitro* citrovorum factor and aminopterin on metabolism of glycine by chick liver homogenates. BARBARA KELLEY (introduced by JOHN R. TOTTER). *Univ. of Arkansas School of Medicine, Little Rock.*

Methylene labeled C^{14} glycine was incubated with liver homogenates of folic acid deficient and control chicks, in the presence and absence of relatively low or high concentrations of citrovorum factor and aminopterin. Specific activities of the glycine and serine isolated by addition of carrier as well as the relative activity found in the β carbon of serine were determined. It was found that in the replete livers there was a 17.5% increase in the specific activity of the serine over that in the deficient livers. This was accompanied by an approximately equal decrease in the glycine counts and a 40% increase in the β carbon counts in the replete livers. Aminopterin added at either low or high levels had no effect on the ratios of the β/α counts of the serine. Low levels of citrovorum factor in deficient chick liver homogenates elevated the ratio of the β/α counts to a value equal to the ratio found in normal controls, whereas a high level of citrovorum factor diminished this ratio by an amount which can be accounted for by the increase in the size of the formate pool. The counts present in the α carbon of the serine (determined by difference) were not markedly altered by either aminopterin or citrovorum factor. Differences in the rate of glycine disappearances will be discussed.

Tracer studies in protein synthesis: antibody formation by spleen slices. ALBERT KESTON AND JEAN-CLAUDE DREYFUS.* *Chemistry Dept. New York Univ. College of Medicine, New York City.*

In experiments where radioactive substances are incubated with tissue slices, radioactivity of protein precipitates is taken to indicate protein synthesis. The usual criteria of isotopic homogeneity are generally inapplicable to these systems. Coprecipitation and firm binding of non-protein substances make such interpretations hazardous. Coprecipitation may occur even on specific precipitates. We have shown that specific precipitates obtained after addition of (presumably irrelevant) pneumococcal polysaccharide S VII and homologous horse antiserum to extracts of rabbit tissues previously incubated with $C^{14}H_2NH_2COOH$, contained considerable radioactivity even after two reprecipitations by CCl_3COOH. On repeating the procedure on the same aliquot additional radioactivity precipitated. Serum globulins do not bind non specific substances appreciably. Systems

involving specific precipitates where antigen and antibody are globulins should be especially favorable for synthesis studies. Spleen and liver slices from rabbits immunized with human or bovine gamma globulin (HGG or BGG) were incubated with $C^{14}H_2NH_2COOH$ and extracts prepared. Equivalent amounts of BGG and homologous antiserum were added to one aliquot from each experiment; similarly the HGG system to another aliquot. Precipitates were washed, dissolved and reprecipitated twice with CCl_3COOH. For the spleen of the HGG immunized animal the HGG precipitates contained several times as much radioactivity as the BGG precipitates while the BGG immunized spleen system showed the reverse. Evidence for appreciable antibody formation by liver was not found.

Estimation of organic compounds as isotopic derivatives using the labeled organic compounds as indicators. ALBERT KESTON AND JOSEPH LOSPALLUTO.* *Chemistry Dept., New York Univ. College of Medicine, New York City.*

The isotopic derivative method (*J. Am. Chem. Soc.* 71: 249, 1949 (1); 72: 748, 1950 (2)) for the analysis of organic compounds requires quantitative conversion of the compound to an isotopic derivative. It has been pointed out (2) that when the organic compound of interest labeled with a second isotope is used as an indicator, quantitative yield is unnecessary. The recovery of the second isotope serves to correct for losses due to incomplete reactions, side reactions, isolations, etc. This method has been applied to the determination of glycine. Known amounts of C^{14}-labeled glycine (A moles) were added to samples containing unknown quantities (X moles) of glycine. The original $C^{14}H_2NH_2COOH$ and the samples were reacted with I^{131} pipsyl chloride without regard for completeness of reaction, and pipsyl glycine purified by paper chromatography (2). Constant C^{14}/I^{131} ratios of successive portions of the chromatograms indicated purity. The C^{14}/I^{131} ratio from the C^{14}-glycine derivative alone divided by this ratio from a sample is equal to $A/(X + A)$. Analysis of amino acid mixtures containing 0.0 and 44.2 μg. showed 0.0 and 45.5 μg. glycine, respectively. For maximum accuracy when filters are used to distinguish the two isotopes, C^{14}/I^{131} should be kept high. Moreover, I^{131}-containing impurities are more easily demonstrated in this situation. This method has an advantage over ordinary isotope dilution procedures in being applicable to minute quantities of materials. Furthermore, non-radioactive impurities do not interfere. The measurement of isotope ratios is simple, accurate, and convenient.

Effect of lowered essential metabolites on growth inhibition produced by 8-azaguanine (guanazolo). G. W. KIDDER, VIRGINIA C. DEWEY AND R. E. PARKS, JR.* *Biological Lab., Amherst College, Amherst, Mass.*

The inhibition of the growth of Tetrahymena produced by 8-azaguanine is decreased when any of a number of metabolites which are dietary essentials for this organism are lowered (by dietary restriction or by the use of structural analogs). The metabolites tested in this system include uracil, thiamine, methionine, phenylalanine, and histidine. Since it has been demonstrated that 8-azaguanine is incorporated into cellular components, (MITCHELL *et al. Cancer Research* 10: 647, 1950) as was earlier predicted in the initial work on Tetrahymena (KIDDER AND DEWEY. *J. Biol. Chem.* 179: 181, 1949), it is probable that restrictions of metabolites reduces 8-azaguanine incorporation and therefore its inhibitory activity. This is further supported by the fact that similar effects are not obtained with combinations of other metabolites—i.e., ethionine and neopyrithiamine.

Phosphorylation reactions of adenosine phosphates in isolated liver mitochondria. W. WAYNE KIELLEY AND RUTH K. KIELLEY (introduced by S. J. THANNHAUSER). *Dept. of Physiology, Tufts College Med. School, Boston, Mass., and Natl. Cancer Inst., Bethesda, Md.*

Some characteristics of the oxidative phosphorylation, dephosphorylation and transphosphorylation reactions occurring in liver mitochondria have been studied. Mitochondria prepared from isotonic sucrose homogenates of mouse liver by a modification of existing procedures neither dephosphorylate nor deaminate adenylic acid. When fresh they possess negligible ATP-ase activity; however this activity is potentially high and is developed by short periods of incubation in absence of oxidizable substrate. This development of ATP-ase activity is prevented during oxidative phosphorylation or by additions of ADP or ATP. The low ATP-ase activity makes possible the nearly complete phosphorylation of adenylic acid during oxidation of α-ketoglutarate. The characteristics of the distribution of the labile phosphate developed during the course of this process can be correlated with the presence of a myokinase, which seems to be present solely in the mitochondria. The maximum rates of the myokinase and oxidative phosphorylation (using α-ketoglutarate) reactions are in a ratio of about four to one. The problem of the primary phosphate acceptor in the phosphorylation process and the significance of myokinase in phosphorylation and dephosphorylation will be discussed.

Inhibition of progestational reactions in uterus of laboratory animals by aminopterin. C. T. G. KING AND JOSEPH T. VELARDO (introduced by E. H. FRIEDEN). *Div. of Labs. and Research, Children's Med. Center, and Children's Cancer Research Fndn., Boston, and Harvard Biological Labs., Cambridge, Mass.*

The typical progestational reactions in the uterus of laboratory animals as induced by progesterone can be inhibited by the simultaneous administration of aminopterin. When 4.5 μg. of aminopterin were injected daily into 20-gm. mice for 28 days with 0.5–1.0 mg. of progesterone, the characteristic effect of the hormone on the uterus was inhibited. The inhibitory action of 4.5 μg. of aminopterin was ineffective when the dose of progesterone was increased to 1.25 mg. daily. The Hooker-Forbes response in the uterus of the mouse to 9 μg. progesterone per ml. was inhibited by 40–50 μg. aminopterin per ml. The decidual reaction in the rat as induced by 1.5 mg. of progesterone daily was partially inhibited by 15–25 μg. of aminopterin, and completely inhibited by 30–50 μg. Abortions in pregnant rats occurred when 0.225 μg. of aminopterin per gm. of body weight was injected twice daily. The progestational reaction in the uterine endometrium of ovariectomized estrous rabbits, as induced by 0.5 or 1.0 mg. of progesterone over a period of 5 days, was completely inhibited by 0.225 μg. of aminopterin per gm. of body weight. The effect of progesterone on the stromal nuclei in the endometrium seems to be inhibited either directly or indirectly by aminopterin. (Supported in part by a grant from the U. S. Public Health Service to Dr. F. L. Hisaw.)

Interaction of arginine and glutathione in beef kidney extracts. JIN H. KINOSHITA* AND ERIC G. BALL. *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

During the course of investigations on glycocyamine synthesis from arginine and glycine as catalyzed by extracts of beef kidney, we have also observed the formation of a product yielding a positive Sakaguchi reaction when arginine and glutathione were the substrates. The reaction mixture is freed of unreacted arginine by treatment with permutit or amberlite resin prior to the application of the color test. Fractionation of kidney extracts has yielded preparations capable of catalyzing the interaction of arginine with glutathione but not with glycine. Glutathione may be replaced by oxidized glutathione but not by glutamine, glutamic acid, or cysteine. Pancreas and kidney are the only tissues of those tried which have yielded the enzyme. The optimum pH for the reaction is 9.0. The product formed has been subjected to paper chromatography using a water-phenol-cyanide mixture as solvent. Identification has been made by use of the Sakaguchi test and an R_f value of 0.65 observed for it. Under similar conditions the R_f value of arginine is 0.46. The compound also reacts with ninhydrin. Separation of the compound by paper chromatography has been followed by extraction and acid hydrolysis. The hydrolysate when chromatographed showed no spot with an R_f of 0.65, but two new spots with R_f values corresponding to arginine and glutamic acid. The observations suggest that the compound is γ-glutamyl arginine formed by a transpeptidation reaction similar to those reported by Hanes *et al.* (*Nature* 166: 288, 1950). Experiments designed to isolate and identify the compound are in progress.

Studies of enzymatically hydrolyzed urinary neutral lipids. RALPH A. KINSELLA, JR., BILLY BAGGETT AND JOHN H. GLICK, JR. (introduced by PHILIP A. KATZMAN). *Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.*

After hydrolysis of urinary neutral lipids by methods previously described (*Federation Proc.* 9: 190, 1950), the yield of formaldehyde on periodic acid oxidation of the water soluble fraction, following benzene-water partition of the residue, was greater than would have been anticipated from the reducing power of the fraction. This discrepancy between reducing power and formaldehydogenicity, with reference to desoxycorticosterone as a standard, was not well accounted for by the properties of Kendall's Compounds B, E or F. Paper chromatography of the water soluble fraction according to the method of Zaffaroni, Burton, and Keutmann yielded large amounts of two components less mobile than Compounds E and F. Alcoholic eluates of these areas possessed no absorption maxima in the neighborhood of 240 mμ and did a maximum at 410 mμ with phenylhydrazine in sulfuric acid by the method of Porter and Silber for 17,21-dihydroxy-20-ketosteroids. Among a number of compounds tested, Compound G, which possesses about one-half the reducing power of DOC, yields one mole of formaldehyde per mole of parent compound, shows about the same mobility on paper chromatograms as the urinary compounds, does not absorb ultraviolet light at 240 mμ, and possesses an absorption maximum at 410 mμ with phenylhydrazine in sulfuric acid. On the basis of this evidence, it is suggested that the tetrahydrocortisone-like compounds, which have been isolated from urine by others, are a quantitatively important fraction of urinary corticoids.

A new procedure for determination of respiration of tissue homogenates. JOHN ESBEN KIRK AND PER FROM HANSEN.* *Div. of Gerontology, Washington Univ. School of Medicine, St. Louis, Mo.*

A procedure has been devised which permits

determination of both the oxygen uptake and carbon dioxide production of tissue homogenates at 20 minutes' interval during an experimental period. The tissue is homogenized in a Pyrex grinder or Latapie mill and the diluted homogenate transferred to a 50- or 100-ml. glass syringe without admission of air. The tip of the syringe is provided with a one-way stopcock. If desired the oxygen content of the medium may be increased by bubbling oxygen through it. During the experiment the syringe is placed in a thermostat. For analysis a sample of the homogenate is transferred to the chamber of a Van Slyke apparatus, and the oxygen, carbon dioxide and nitrogen content determined by the method of Van Slyke and Neill. Five ml. of an acid ferricyanide solution are first evacuated in the apparatus and the extracted gas ejected; 5 ml. of homogenate are then introduced from the syringe through the left capillary of the extraction chamber, the exact measuring of the sample being accomplished by means of the 10-ml. graduation mark. During the transfer of the sample contact with air is prevented by use of a ball and socket glass joint. The syringe procedure also makes possible the withdrawal at any time of homogenate samples for analysis of other metabolites (e.g. lactic acid). The syringe is easy to sterilize and sterility can be maintained throughout an experiment by covering the syringe with a mantle of cellophane paper.

Relation of coenzyme A to steroid and total lipid synthesis in yeast. HAROLD P. KLEIN (introduced by CLAUDE A. VILLEE). *Biochemical Research Lab., Massachusets General Hosp., Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

By incubation of resting pantothenic acid-deficient yeast, with and without pantothenic acid, comparable samples of coenzyme A (Co A)-poor and Co A-rich yeast were obtained. For studies on lipid synthesis, such resting suspensions were incubated in acetate medium for 16 hours, after which the cells were dried and extracted with chloroform for total lipids. The ergosterol content of the non-saponifiable fraction was determined spectrophotometrically. It was found that with Co A-poor samples, ergosterol and lipid synthesis was depressed or nearly abolished. For instance, in 4 experiments, yeast samples containing an average of only 56 U of Co A per gram cell, synthesized 0.6 mg. of ergosterol and 1.6 mg. of total lipid per gram cell. In contrast, yeast containing 260 U of Co A per gram cells, synthesized 4.1 mg. ergosterol and 10.9 mg. of total lipid per gram cell. With Co A-rich yeasts, total lipids increased about 130% and steroids 150%, while in Co A-poor cells, total lipids and steroids increased only by 21%. The data suggests that the conversion of acetate to steroid and to lipid, in general, occurs through Co A-catalyzed reaction systems.

Effect of selenium on activity of certain enzymes in homogenates of rat tissues. HARLAN L. KLUG,* DONALD F. PETERSEN* AND ALVIN L. MOXON. *Chemistry Dept., South Dakota Agricultural Experiment Station, Brookings.*

Following the demonstration of an *in vivo* inhibition of rat liver succinic oxidase, an extensive *in vitro* study of this system was done to determine the mechanism of the inhibition. The locus of the action is found to be succinic dehydrogenase. The order of addition of succinate affects the extent of the inhibition and studies with malonate and selenite show these two compounds to be mutually exclusive inhibitors. Using the delayed substrate technique, the inhibition is found to be an interference or irreversible type in which selenium apparently titrates the succinic dehydrogenase. The sulfhydryl concentration of the inhibited system is decreased as is the selenium content of the supernatant from the trichloroacetic acid precipitated flask content. Reduced glutathione does not prevent inhibition or restore the decreased oxygen uptakes of inhibited systems. The percentage of *in vitro* inhibition with 4×10^{-5} M selenite approximates 50% each for liver, kidney, brain, and lung tissues but toxic intraperitoneal and subcutaneous injections show brain and lung more sensitive than liver or kidney. *In vitro* studies show that liver malic dehydrogenase, fumarase, and adenosine triphosphatase are not sensitive to selenium while oxalacetic acid oxidase and choline oxidase are inhibited. Nitrogen balance, and xanthine oxidase studies indicate no interference with protein absorption by selenium.

Fate of C^{14} labeled acetyl salicylic acid. R. W. KNOUSE,* W. D. PAUL* AND J. I. ROUTH. *Dept. of Biochemistry and Medicine, State Univ. of Iowa College of Medicine, Iowa City.*

Sodium acetate-1-C^{14} was converted into acetyl chloride which was reacted with salicylic acid to produce acetyl-1-C^{14} salicylic acid. A 200-mg/kg. dose of labeled aspirin was administered orally to a rat. After 3 hours the radioactivity of the organs, urine and expired CO_2 was determined. Total activity was greatest in intestine, muscle, blood and kidney cortex, low in excreted urine. Approximately 40% of administered radioactivity was found in the expired CO_2 indicating considerable cleavage and oxidation within 3 hours. After administration to rabbits, plasma salicylates and radioactivity of plasma, urine and expired CO_2 were followed. The salicylate level rose for 40 minutes after oral administration, then leveled off, while radioactivity reached a maximum earlier and leveled off. Following intravenous adminis-

tration, levels of both salicylate and radioactivity were highest immediately and gradually fell, with radioactivity decreasing more rapidly. Studies on the rate of hydrolysis of aspirin are in progress. After 90 minutes 10% or less of the administered radioactivity was found in the expired CO_2, with the highest values after intravenous administration. The radioactivity of the urine was low after oral administration, but increased severalfold following intravenous injection, with maximum excretion after 10 minutes. Preparation of acetyl carboxyl-C^{14}-salicylic acid is in progress.

Purine metabolism during bacteriophage growth. ARTHUR L. KOCH (introduced by E. A. EVANS, JR.). *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Purine metabolism in *E. coli* multiply infected with bacteriophage T_6 has been studied by use of radioactive purines. The purines were prepared biosynthetically by growth of *E. coli* on lactate medium containing $NaHC^{14}O_3$. The purines were isolated chromatographically using Dowex-50 columns. 94% of the activity of the guanine was found in carbon 6. Bacteria were labeled specifically in their purines by growth on lactate medium supplemented with radioactive adenine or guanine. Interconversion of the purines occurred. With bacteria grown with small amounts of labeled adenine, the cells contained the same activity in both purines. Phage grown on the washed, resuspended cells obtained 14% of their adenine and 20% of their guanine from the host. With bacteria grown with large amounts of labeled guanine, the cells contained three times as much activity in the guanine as in adenine but phage grown on the washed, resuspended cells obtained 21% of their adenine and 29% of their guanine from the host. Apparently the medium purines are interconverted in host growth but preformed bacterial purines are not interconverted in phage reproduction. Infection of unlabeled bacteria in the presence of radioactive guanine gave phage in which the activity of the adenine was 23% that of the guanine over a wide range of guanine concentration in the medium. Therefore, interconversion of medium purines goes on in the infected system as well as in normal bacteria but purine transfer from the host to the phage probably does not go via the free bases.

Variation in solubility of cholesterol from different sources at low temperature. ALFRED E. KOEHLER AND ELSIE HILL.* *Sansum Clinic Research Fndn. and Santa Barbara Cottage Hosp., Santa Barbara, Calif.*

Solubility of serum lipides in organic solvents at low temperatures permits separations otherwise not easily accomplished. The solubility curves for free cholesterol, esters and neutral fats in methanol, ethanol and petroleum ether at temperatures 0° to −60° have been established. At −60° in methanol, free cholesterol can be separated from the esters and to a large extent from neutral fats. Free cholesterol from different sources so prepared, however, has different solubilities. A highly repurified commercial cholesterol (spinal cord) had a solubility of 2.5 mg/10 cc methanol at −60° while gallstone cholesterol was 3.8 mg., red cell cholesterol was 4.7 mg. and serum cholesterol was 4.5 mg. Digitonin precipitation and regeneration of the various cholesterols did not appreciably alter their solubilities. Such precipitated cholesterol, however, still contains some non-chromogenic substances and cannot be regarded as a pure single substance and such contaminants could conceivably alter solubility. However, control determination with the addition of various vegetable oils and fats (cholesterol free) and pure fatty acids, from butyric to oleic acid, to commercial cholesterol did not appreciably alter its solubility. There are indications that serum cholesterol can be separated by solubility into two or more fractions. In as much as commercial cholesterol solubility differs more widely from the other preparations studied it must be remembered that it has undergone ten recrystallizations and therefore the end product could represent a more insoluble form than the original.

Enzymes of oxidative decarboxylation of pyruvate. SEYMOUR KORKES,* ALICE DEL CAMPILLO* AND I. C. GUNSALUS. *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

Two enzyme fractions (*a* and *b*) have been obtained by ammonium sulfate fractionation of *E. coli* extracts which, in presence of Mg^{++}, DPN, coenzyme A (Co A), transacetylase (E. R. STADTMAN, *Federation Proc.* 9: 233, 1950), and lactic dehydrogenase, catalyze reaction 1 (2 pyruvate + phosphate → acetyl phosphate + CO_2 + lactate). This is the sum of reactions 2 (pyruvate + phosphate + DPN_{ox} → acetyl phosphate + CO_2 + DPN_{red}) and 3 (pyruvate + DPN $\underset{red}{\rightleftharpoons}$ lactate + DPN_{ox}). Reaction 3 is catalyzed by lactic dehydrogenase, reaction 2 by at least three enzymes, *a*, *b*, and transacetylase. There is no reaction in the absence of either orthophosphate, DPN, Co A, transacetylase, enzyme *a*, or enzyme *b*. A reaction occurs in the absence of transacetylase if crystalline condensing enzyme (*Nature* 166: 403, 1950) and oxalacetate are added. This gives reaction 4 (2 pyruvate + oxalacetate → citrate + CO_2 + lactate). Similar results were previously obtained with crude *E. coli* extracts (which contain transacetylase) in the absence of orthophosphate (*Nature* 166: 439, 1950). The above indicates that pyruvate reacts as follows: 2 pyruvate

+ Co A → acetyl-Co A + CO_2 + lactate, and that, with catalytic amounts of Co A, little reaction occurs unless Co A is regenerated by either reaction 5 (acetyl-Co A + phosphate ⇌ acetyl phosphate + Co A), or 6 (acetyl-Co A + oxalacetate ⇌ citrate + Co A). Reaction 5 is catalyzed by transacetylase, reaction 6 by the condensing enzyme.

Kinetics of peptide bond formation. D. E. KOSHLAND, JR. (introduced by JOHN T. EDSALL). *Dept. of Chemistry, Harvard Univ., Cambridge, Mass.*

The acyl phosphates have been suggested as intermediates in protein synthesis (LIPMANN, *Advances Enzymal.* 1: 99, 1941) and in amide bond formation, e.g. in the formation of glutamine (SPECK, *J. Biol. Chem.* 179: 1405, 1949). To aid in clarifying the mechanisms of these reactions, the acetylation of some amines and amino acids by acetyl phosphate has been studied kinetically in aqueous solutions. The decrease in acetyl phosphate concentration in buffered solutions at 39.00° C. was measured by a modification of the hydroxamic acid test of Lipmann and Tuttle (*J. Biol. Chem.* 159: 21, 1945). The acetylation occurs at a moderate though measurable rate at pH = 7 in the absence of any enzymes and is strongly base catalyzed. Some representative second order constants at pH = 7.3 in liters moles^{-1} minutes^{-1} are: glycine, 0.011; glycylglycine, 0.036; ammonia, 0.002. The first order constant for hydrolysis under these conditions is 0.0039 minutes^{-1}. From the relative rates of hydrolysis and acylation, it is possible to exclude a non-enzymatic acylation in glutamine formation and to indicate the improbability of a similar non-enzymatic step in protein synthesis. The observed kinetics also suggest some properties of the active portion of the postulated enzyme surface for enzymatic acylation of amino compounds.

Relationship between parent infecting virus and virus progeny. LLOYD KOZLOFF (introduced by H. S. ANKER) *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Bacteriophage T_6r^+ was labeled by growth on *E. coli* in synthetic culture medium containing $N^{15}H_4Cl$ (62 atom % excess) as the source of nitrogen. The uniformly labeled virus was concentrated and purified by differential centrifugation until the virus preparation was free of non-viral N and was homogeneous in the ultracentrifuge. Bacteria in isotope free synthetic medium were infected with this labeled parent phage producing a single generation of virus progeny. In agreement with earlier experiments using P^{32} labeled parent phages only a small fraction (10–30%) of the N of the infecting virus particle was found in the virus progeny. The bulk (50–70%) of the isotopic N of the parent virus nucleoprotein was found as non-sedimentable N in the lysate. 10–18% of the N^{15} was actually present in the form of NH_3 in this fraction. The concentration of N^{15} in the progeny desoxyribonucleic acid was always higher (several-fold) than the concentration of N^{15} in the progeny protein indicating a greater conservation of parent nucleic acid than of parent protein during virus reproduction. Experiments were performed in which prior to infection the labeled phage preparation was 99.9% inactivated by irradiation with either ultraviolet light or x-rays. When bacteria were infected with mixtures of these irradiated labeled phage and live unlabeled phage the amount of N^{15} found in the progeny in both cases was equal to that found in the control experiment. This offers the first direct chemical evidence that irradiated dead phage do participate in viral reproduction.

Reactivation of norite-treated zymase with muscle glyceraldehyde-3-phosphate dehydrogenase as a source of DPN. EDWIN G. KREBS (introduced by EARL R. NORRIS). *Dept. of Biochemistry, Univ. of Washington School of Medicine, Seattle.*

It has been shown by Taylor *et al.* that DPN bound to crystalline muscle glyceraldehyde-3-phosphate dehydrogenase can react in the lactic dehydrogenase system. In the present study it was found that DPN in the same form can serve to reactivate zymase from which the DPN has been removed with norite. Zymase activity was followed by measuring CO_2 production with glucose as a substrate. With the norite-treated zymase, the concentration of added DPN needed to give half maximal activation was between 4 and 6 x 10^{-6} M. This value was the same regardless of whether free DPN was added or whether the crystalline muscle enzyme with known DPN content was used. These results suggested that the bound DPN is available for the yeast alcohol dehydrogenase system. Further data substantiates this. Kinetics studies with bound and free DPN in the alcohol dehydrogenase reaction will be reported.

Availability of amino acids in cottonseed products. K. A. KUIKEN. *Texas Agricultural and Mechanical College System, College Station.*

The availability of the essential amino acids in cottonseed meal was studied by a technique which involved feeding rats and determining the amount of the amino acid consumed and the amount excreted by way of the feces. Marked variations in availability of the individual amino acids in commercial hydraulic type products were observed. Although 95% of the arginine in such samples was available to the animals, only 65%

of the lysine was available. Amino acid availabilities above 90% were obtained with a solvent extracted product of low gossypol content which had been prepared without heat. The availabilities of the amino acids in this product were not altered seriously by heat treatments ranging in severity to an extreme of autoclaving at 15 lb. pressure for one hour. The addition of 0.1 % gossypol, as the pure compound or the equivalent amount of pigment glands, did not reduce amino acid availabilities appreciably even though mild heat treatment was used to effect binding of the gossypol. A raw hexane extracted product containing 1.2% free gossypol was found to have an arginine availability of 96% and a lysine availability of 84%. Steaming for 30 minutes or autoclaving for 10 minutes reduced lysine availability to 80%. Observations on the effect of heating cottonseed meal in the presence of cottonseed oil and on the effect of feeding a gossypol-lysine addition compound will be reported.

Transamination of tyrosine by rat liver acetone powder. BERT N. LADU, JR. AND MARVIN FINK. (introduced by BERNARD B. BRODIE). *New York Univ. Research Service, Goldwater Memorial Hosp., New York City, and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

It has been observed (LADU, B. N. AND D. M. GREENBERG, *J. Biol. Chem.* In press) that an extract of an acetone powder of rat liver contains a series of enzymes which catalyze the oxidation of L-tyrosine to acetoacetic acid. *p*-Hydroxyphenylpyruvic acid and homogentisic acid were also found to be converted to acetoacetic acid by this preparation. Additional evidence is presented for the view that the first step, the conversion of tyrosine to *p*-hydroxyphenylpyruvic acid, is a transamination rather than an oxidative deamination. The evidence for this is *1*) manometric data are in agreement with a transamination step, *2*) considerably less free ammonia is produced than would be expected from an oxidative deamination reaction, *3*) no L-amino acid oxidase activity is present under the experimental conditions used, *4*) the rate of tyrosine oxidation is accelerated by the addition of α-keto glutarate, *5*) the reverse step, the formation of tyrosine from *p*-hydroxyphenylpyruvic acid and glutamic acid, can be demonstrated with the preparation under anaerobic conditions. Pyruvate, and oxalacetate in high concentrations, inhibit tyrosine oxidation. The addition of pyridoxal phosphate does not stimulate the rate of oxidation under the experimental conditions used. Work is in progress to concentrate the transaminase activity.

Interaction of spca with platelets or thromboplastin in prothrombin conversion. GRETA LANDWEHR AND BENJAMIN ALEXANDER (introduced by RALPH W. MCKEE). *Beth Israel Hosp. and Harvard Med. School, Boston, Mass.*

A study is presented of the role of platelets, thromboplastin, and spca in prothrombin conversion. Human platelets were processed (0°C.) from citrated blood collected with Arquad (Armour) coated needles in siliconized glassware, washed thrice with two plasma volumes of saline, and resuspended in saline. Platelets plus 0.0025 M $CaCl_2$ convert purified human prothrombin (citrate free), prepared by $BaSO_4$ adsorption and citrate elution, to thrombin slowly. The conversion curve suggests an autocatalytic reaction. When purified human spca (thrombin free) is incorporated in the mixture, thrombin formation is markedly accelerated. Preincubation of platelets with spca (30-60 minutes, room temp.) induces even more rapid conversion. Furthermore, this prior incubation obviates the autocatalytic nature of the curve. Spca plus platelets, or spca plus prothrombin alone yielded no thrombin, and thrombin contaminants of the prothrombin never exceeded that amount which could clot the standard fibrinogen solution in 2800 sec. The same phenomena were observed when purified bovine lung thromboplastin replaced platelets. The ability of platelets to convert prothrombin is, within certain limits, directly related to the amount of platelets. Their 'thromboplastic' activity per U of nitrogen compares favorably with bovine lung thromboplastin, and remains unaltered at 4°C. for at least 8 days. These experiments indicate *1*) that both platelets and thromboplastin interact with spca, and *2*) platelets contain considerable thromboplastic activity. Thus, three stages in coagulation have been demonstrated with human preparations obtained exclusively from blood. The resemblance of spca to thrombokinase (Milstone) in its prothrombin-converting property is striking.

Oral administration of vitamin B_{12} containing cobalt^{60} to rats. C. A. LANG* B. F. CHOW, C. ROSENBLUM,* R. H. SILBER, D. T. WOODBURY* AND R. YAMAMOTO.* *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

The microbiological activity of B_{12} given orally to rats or man appears largely in the stool and little in the urine. Therefore, B_{12} so administered might result in the conjugation to a form unavailable for the microorganism, poor absorption in the intestinal tract, or conversion to a form incapable of being excreted by the kidneys. The availability of B_{12} containing radioactive cobalt^{60} permits a differentiation between the administered vitamin and that originally present, assuming that radioactivity due to cobalt^{60} represents B_{12}. Twenty-four-hour collections of urine and stool of 4 normal

rats were made 2 days prior to and 3–4 days after, the oral administration of 0.89 mg. (0.18 mc.) of radioactive B_{12}. After the last collection the animals were bled completely and certain organs removed and homogenized. Organ homogenates, blood, urine, and stool were assayed for both radioactivity and microbiological activity. The determinations showed that significant amounts of radioactivity were found in blood, liver, and kidney. Of the vitamin administered a major portion appeared in the feces and a minor fraction in the urine. Distribution experiments using immiscible solvents followed by microbiological assay and radioactivity was most probably due to B_{12} and not to free inorganic cobalt. Hence B_{12} given orally is absorbed, though poorly, and appears largely in the stool and that B_{12} molecule is not broken down to any appreciable extent.

Enzymatic analysis of structure of branched polysaccharides. JOSEPH LARNER,* BARBARA ILLINGWORTH* AND GERTY T. CORI. *Dept. of Biochemistry, Washington Univ. Med. School, St. Louis, Mo.*

The structures of rabbit liver and muscle glycogen and of corn and wheat amylopectin have been investigated by first allowing crystalline muscle phosphorylase to act exhaustively on the parent polysaccharides in a medium containing inorganic phosphate. The limit dextrins so formed were isolated by several precipitations with ethanol and submitted to the action of amylo-1,6-glucosidase (*J. Biol. Chem* vol. 188, 1951) in a phosphate-free medium until the reaction had come to an end point. The glucosidase was inactivated by heating, the phosphorylase step repeated and the second limit dextrins isolated. These separate degradations were repeated until 70–90% of the original polysaccharides had been digested. Models were constructed based on the amount of phosphorylated glucose obtained after phosphorylase, and of free glucose after amylo-1,6-glucosidase action. As a further aid in structure elucidation, separate aliquots of the limit dextrins were analyzed for percent end-groups and also submitted to the action of crystalline β-amylase. The polysaccharides were found to differ in the length of the outer and inner chains and in the number of tiers of branch points. The analytical results obtained indicate that glycogen and amylopectin have an irregular, tree-like, and not a laminated or comb-like structure.

In vivo dialysis as a means of influencing survival of x-irradiated dogs. PAUL S. LAVIK,* JACK R. LEONARDS, CHARLES R. HEISLER* AND G. WARREN BUCKALOO.* *Dept. of Biochemistry and Atomic Energy Med. Research Project, Western Reserve Univ., Cleveland, Ohio.*

In the present investigation we have explored the possibility that dialyzable substances, having toxic properties, are formed and/or accumulate in the body under the influence of ionizing radiation and are responsible for its lethal effects. Eight adult mongrel dogs were connected via the femoral artery and vein to an artificial kidney (SKEGGS, LEONARDS AND HEISLER *Proc. Soc. Exper. Biol. & Med.*, 72: 539, 1949) and their blood dialyzed continuously during and for 1.5–2.5 hours after the administration of 550 roentgen whole-body x-irradiation (6.2 r/minutes, 220 kv., 9 ma., 1 mm. Al, 0.5 mm. Cu). Six dogs died between 2 and 21 days after irradiation; two were still alive after 8 and 30 days, respectively. These results differ little from the mortality rate found for 10 control dogs similarly irradiated but not dialyzed, i.e., 8 out of 10 of these animals died between 5 and 25 days after irradiation while the other 2 were still alive at 90 days. The sharp fall in the total white blood cell count during the first week after irradiation to values of less than 1000 per cu. mm. was also unaffected by the dialysis procedure. Preliminary results of other experiments, in which the dialysis was begun at various times after irradiation, were also without beneficial effects.

Decomposition of methionine by hot sulfuric acid. THEODORE F. LAVINE AND NORMAN F. FLOYD.* *Lankenau Hosp. Research Inst., Philadelphia, Pa.*

The relatively low yield of homocystine obtained from the decomposition of methionine in hot 50% sulfuric acid (BUTZ, L. W., AND V. DU VIGNEAUD. *J. Biol. Chem.* 99: 135, 1932–33) suggests that disulfide formation is only one of several simultaneous reactions under these conditions. Experimental evidence indicates that aside from homocystine, the other major reaction products are methionine methyl sulfonium sulfate and sulfur dioxide. The sulfonium salt was isolated as the bromide. On the basis of the data obtained, the course of the reaction may be described by the following equations:

$$2\ RSCH_3 + H_2SO_4 \rightarrow 2\ RSH + (CH_3)_2SO_4$$
$$2\ RSCH_3 + (CH_3)_2SO_4 \rightarrow (RS(CH_3)_2)_2SO_4$$
$$2\ RSH + H_2SO_4 \rightarrow RSSR + 2H_2O + SO_2$$

$$4\ RSCH_3 + 2H_2SO_4 \rightarrow RSSR + (RS(CH_3)_2)_2SO_4 + 2H_2O + SO_2$$

Thus, only half of the methionine is available for disulfide formation. This mechanism is analogous to that proposed by Haas and Dougherty (*J. Am. Chem. Soc.* 65: 238, 1943) to account for the decomposition of benzyl sulfide by hot sulfuric acid. Details of the several steps and the use of sulfuric acid as an oxidizing and alkylating medium will be discussed.

Cortisone and sulfate metabolism of animal tissues. LAURENCE L. LAYTON (introduced by PHILIP L. HARRIS). *Johns Hopkins Univ., Baltimore, Md.*

It has been shown (*Cancer* 3: 725, 1950; *Proc. Soc. Exper. Biol. & Med.* 73: 570, 1950) that sulfate ion is absorbed and fixed in an insoluble organic form by embryonic tissues and by the regenerating tissue from healing wounds. Since cortisone has been reported to have an inhibitory action on wound healing, and since we had found that sulfate is utilized in the synthesis of the connective tissue substance, it appeared probable that cortisone would affect the fixation of sulfate by tissues. Tissue fragments from the aortic arch, heart, skeletal muscle, and cartilage of embryonic rats and chicks, and regenerating tissue from healing muscle wounds of chickens were maintained *in vitro* in a survival medium containing labeled inorganic sulfate ion and various levels of cortisone alcohol. It was found that cortisone acted to inhibit the fixation of sulfate. A graded inhibitory response was obtained from different levels. High concentrations of cortisone alcohol completely suppressed sulfate fixation without affecting sulfate conjugase (sulfate esterification) activity or the migration of fibroblasts in fresh tissue explants during the first 48 hours. A partial explanation is suggested for the action of cortisone in the therapy of certain connective tissue diseases.

Effect of anoxia on metabolism of myocardial tissue. JANET M. LEMLEY AND GEORGE R. MENEELY (introduced by CHARLES S. ROBINSON). *Radioisotope Unit, Thayer Veterans Administration Hosp. and Depts. of Biochemistry and Medicine, Vanderbilt Univ., Nashville, Tenn.*

The extreme sensitivity of cardiac muscle to anoxia is well established. In these experiments the oxygen uptake was measured in homogenized myocardial tissue from rats exposed to acute anoxia produced in a low-pressure chamber. The animals were subjected to an atmosphere equivalent to 7% oxygen for one hour and killed by exposure equivalent to 3% oxygen. At the moment of respiratory arrest the hearts were removed, homogenized in buffered salt solution and oxygen uptake measured. It was found that homogenized myocardial tissue from rats exposed to anoxia used only one-half as much oxygen as homogenates from normal animals. The Q_{O_2} for the control group was 4.06 ± 0.0335 as compared with a Q_{O_2} of 2.15 ± 0.0335 for the experimental group. The oxygen consumption of homogenates from anoxic hearts was increased threefold by the addition of an extract of boiled myocardial tissue. Boiled heart extract from both normal and anoxic animals produced this increase but the former was slightly more effective.

Removal of fluid from normal and edematous dogs by means of ultrafiltration. JACK R. LEONARDS, LEONARD T. SKEGGS, JR,* AND JOSEPH R. KAHN.* *Dept. of Biochemistry and Pathology, Western Reserve Univ., and Veterans Administration Hosp., Cleveland, Ohio.*

Blood obtained from the femoral artery of dogs was made to flow in a thin film (about 1 mm.) between two sheets of cellophane and returned to the animal via the femoral vein. The cellophane sheets (12 in. x 18 in.) were supported on either side by corton cloths and grooved rubber pads. Ultrafiltration was effected by evacuating the spaces between the cellophane and the grooved rubber pads. Four such units held 350 ml. of blood and the rate of ultrafiltration was up to 600 ml. per hour. The procedure itself has no harmful effects on normal dogs if the fluid removed is replaced by a suitable salt solution. Up to 2500 ml. has been exchanged in this manner. Four normal dogs all died following the removal of 700–1240 ml. of fluid (5.2–7.6% of body weight) at rates of 2.3–8.5 ml/minute. The blood of these animals became very concentrated with respect to red blood cells and proteins, while the electrolyte composition of the plasma and ultrafiltrate remained essentially normal. The introduction of Dakin's solution in the pericardium of 4 dogs produced a constrictive pericarditis which after several weeks resulted in severe ascitis and some generalized edema. No more fluid could be removed from these dogs than from the normals before death resulted. Marked edema was obtained in 3 dogs as a result of the hypoproteinemia produced by a low protein diet and plasmaphoresis. Ultrafiltration at rates of 5.2-6.7 ml/minute resulted in death of these dogs when 1800–2100 ml. of fluid had been removed, although the animals had a minimum of 500 ml. of edema fluid at the start of the experiment. Harmful effects, evidenced by a fall in blood pressure, were noted after removal of as little as a liter of fluid. Under these conditions, the amount of edema fluid which could be safely removed by ultrafiltration was quite limited.

Incorporation of ethionine into rat proteins. MELVIN LEVINE (introduced by HAROLD TARVER). *Univ. of California Div. of Biochemistry, Berkeley.*

Rats were injected intraperitoneally with ethionine containing C^{14} in the methylene carbon of the ethyl group and were sacrificed at various time intervals thereafter. About 70% of the activity was excreted in the urine whereas less than 3% was excreted as respiratory carbon dioxide in 24 hours. Determination of activities of protein samples (material precipitable by TCA) showed significant uptake of C^{14} which could not be re-

duced by washing the proteins with TCA, monothioethyleneglycol, by solution in NaOH-urea and reprecipitation, or, in a few cases, by solution in NaOH-urea containing ethionine carrier and dialysis. In general the uptake of activity in the tissues (plasma, spleen, kidney, liver, and intestinal mucosa) followed the same order found with serine, glycine, and methionine. Carrier experiments with ethionine and methionine and a conversion of the S-alkyl groups to the isothiourea picrates indicated that a large fraction of the activity was present as ethionine. No radioactivy was detected in the methionine. The non-protein activity in the fresh tissues and the ethionine activity in the proteins when compared with the results found in *in vitro* studies on rat plasma indicated that the ethionine found in the tissue proteins could not be accounted for by adsorption. The decrease with time of activity in the proteins due to ethionine was much more rapid than that found with serine, glycine and methionine. The results are compatible with the view that ethionine is incorporated into the tissue proteins in the rat.

Purification of acetylcholinesterase. SUMNER LEVINE (introduced by DAVID SHEMIN). *Dept. of Neurology, Columbia Univ., College of Physicians and Surgeons, New York City.*

The procedure previously described for the purification of acetylcholinesterase from electric tissue of *Electrophorus electricus* suffers from the disadvantage of requiring much time to obtain a preparation of high purity. A method has been developed using calcium phosphate gel as an absorbent. After subsequent elution the material is fractionated with $(NH_4)_2SO_4$ at controled pH. A material of high purity was obtained. Studies have been made of the homogeneity of this material using the ultracentrifuge and electrophoretic technique. The nature of the active site has been investigated employing various group specific reagents.

Reaction of pyridoxal with thiophene. VICTOR E. LEVINE AND WARREN G. HANSEN.* *Dept. of Biological Chemistry and Nutrition, Creighton Univ. School of Medicine, Omaha, Nebr.*

Thiophene reacts with numerous carbonyl compounds in the presence of sulfuric acid to yield colored complexes. Short-chain aliphatic aldehydes and ketones of physiologic significance (acetaldehyde, glyceraldehyde, pyruvic aldehyde, acetoacetic ester, dihydroxyacetone, pyruvic acid, phenylpyruvic acid) react to yield complexes generally of red to orange color. Acetone, α-ketoglutaric acid and oxaloacetic acid do not react. Pyridoxal or pyridoxal phosphate in aqueous solution reacts with a 0.5% alcoholic solution of thiophene and 80% sulfuric acid to yield a colored complex of a very stable jade-green color. Other vitamin B_6 derivatives, pyridoxine, pyridoxamine and pyridoxic acid, do not yield a colored complex. The thiophene reaction is therefore a rapid and simple means of distinguishing between pyridoxal and other vitamin B_6 derivatives. The reagents and the compound being tested are mixed at 0°C. The reaction mixture is subsequently heated in the water bath at 75°C. until maximum color develops. Spectrophotometric studies show that a characteristic absorption peak at 615 mμ is obtained by the reaction of either pyridoxal or pyridoxal phosphate with thiophene and sulfuric acid. Calibration curves have been prepared at this wave length, and it has been found that the reaction is quantitative, and that the Beer-Lambert law is followed at concentrations up to 100 μg. per ml. The thiophene reaction may serve as a practical method for the estimation of the pyridoxal content of tissues and biologic fluids.

Hydroxy amino acids in tissue filtrates. GEORGE T. LEWIS, LILLIAN HANKEY,* FOSTER ADAIR* AND MARTIN CUMMINGS.* *Dept. of Biochemistry, Emory Univ. School of Dentistry, Emory University and Tuberculosis Section, Lawson Veterans Administration Hosp., Chamblee, Ga.*

In an attempt to elucidate some problems of host resistance, tungstic acid filtrates of the lungs, liver and spleen of rats, guinea pigs and rabbits have been analyzed for hydroxy amino acids by periodic acid oxidation, for amino nitrogen by a modification of the Folin colorimetric procedure and for total nitrogen by micro-Kjeldahl. The results indicate only minor variations between species. The liver contains as yet unidentified, non-nitrogenous material which gives formaldehyde under the influence of periodic acid. The substance does not seem to be a carbohydrate. Intraperitoneal injection into rats of an irritant such as mineral oil causes an increase in the hydroxy amino acid levels in a remote organ, the lungs. The content of the tissue in amino acid nitrogen is much less markedly affected and the total nitrogen of the tungstic acid filtrate remains virtually unchanged. Other agents which induce an inflammatory reaction, for example BCG and heat-killed tubercle bacilli, also bring about an increase in the hydroxy amino acid fraction. Cortisone has an effect on the hydroxy amino acid and amino nitrogen levels and, in this connection, the relation between adrenal cortical function and host resistance will be discussed. Chromatograms show a different pattern for the extracts of organs from animals in which an inflammatory response has been induced and also following the administration of cortisone. However, the chromatographic technique has not been helpful in an

identification of the periodic acid-reacting substance in liver.

Paper chromatography of fatty acids. L. A. LIBERMAN,* ALEJANDRO ZAFFARONI* AND ELMER STOTZ. *Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Recent publications by Hiscox and Berridge (*Nature* 166: 522, 1950) and Brown and Hall (*Nature* 166: 66, 1950) describe a method for the paper chromatography of the lower and intermediate fatty acids. Their procedures employ small amounts of the acids as salts of a volatile base, such as ammonia or ethyl amine, using butanol as the mobile solvent. Positions of the compounds are revealed by acid-base indicators. In attempting to apply this method to the higher fatty acids, it was found that they moved almost with the solvent front and with streaking. This method has now been modified so that the higher saturated fatty acids, up to stearic acid, can be chromatographed and, in some cases, separated from a mixture. The salient features of the modification are *1*) the use of paper impregnated with paraffin as the stationary phase, *2*) employment of pentanol, henanol, or 'octyl alcohol' as the organic phase and *3*) substitution of methyl amine or the strongly basic diethyl amine as the volatile base. In such a system (ascending chromatogram) lower and intermediate fatty acids have much smaller Rf values and improved resolution compared to the previous method. The higher acids, such as palmitic or stearic exhibit concise spots with little or no tailing. In mixtures, lower fatty acids can be separated from the higher ones, but the higher acids have a tendency to run together and, therefore their development is obscure.

Steroid excretion after administration of ACTH in man. SEYMOUR LIEBERMAN, LUCIE B. HARITON,* M. B. STOKEM,* P. E. STUDER* AND KONRAD DOBRINER. *Sloan-Kettering Inst. for Cancer Research, New York City.*

The steroid excretion patterns before, during, and after the administration of ACTH and cortisone acetate to human subjects have been further investigated. (*Federation Proc.* 9: 196, 1950). In the 'water soluble' fractions in addition to compounds E, F, and 3α, 17α, 21-trihydroxypregnane-11,20-dione we have also identified 3α, 11β, 17α, 21-tetrahydroxypregnan-20-one as the diacetate m. p. 235–238°; $(\alpha)D = +89 \pm 2$ (acetone). From the 'Benzene Soluble' fraction, two new steroids have been identified by means of infrared spectrometry and were isolated and characterized as the acetates. These were 3α, 17α-dihydroxypregnane-11,20-dione (mono acetate m.p. 194–195°) and 3β, 21-dihydroxy-Δ^5-pregnen-20-one (diacetate m.p. 157–164°). The metabolic significance of these compounds and their precursors will be discussed.

Observations on some biochemical changes induced by administration of vitamin B_{12} to deficient animals. CHIUN T. LING* AND BACON F. CHOW. *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

Although our knowledge of the metabolic role of vitamin B_{12} is meager, experimental results point to its possible relationship to carbohydrate or fat metabolism. In order to throw some light on this subject, experiments were performed so as to follow some biochemical changes induced as a result of administering vitamin B_{12} to deficient animals. Rats from parents raised on soybean diet were used in all experiments. Each series of experiments consisted of a deficient and a B_{12}-injected group. Vitamin B_{12} was given subcutaneously to the latter in dosages ranging from 25 milligamma/day to 0.5 gamma 3 x a week for as long as 12 weeks. During the period of injection, blood sugar levels after varying period of fasting, were determined on both groups of animals. Blood sulfhydryl content was measured by the amperometric titration method. At the end of the experimental period, the animals were bled by cardial puncture and sacrificed. Glycogen, fat, and nitrogen content of the liver, and total nitrogen, fat, and muscle glycogen content of the carcasses were analyzed. The results show that the deficient animals had much lower body-fat, and higher water content, and lower blood sulfhydryl content, as compared to their litter mates on the same diet but receiving B_{12}-injection. The significance of these findings is discussed in the light of the role of vitamin B_{12} in carbohydrate, fat, and protein metabolism.

Oxidation of nitroethane by extracts fron Neurospora. HENRY N. LITTLE (introduced by VICTOR A. NAJJAR). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Extracts from *Neurospora* were found to catalyze the oxidation of nitroethane to yield nitrite and aldehyde with a concurrent consumption of oxygen. The enzyme catalyzing this has been purified about twentyfold by ammonium sulfate fractionation and some of the properties of the partially purified enzyme have been studied. No dialyzable co-factor has been found necessary for enzyme action. Optimum activity was obtained at pH 7.0 with 0.05 M nitroethane as substrate. Nitropropane, and to a slight extent, nitromethane were also acted on by the enzyme. Cyclic nitro compounds did not appear to serve as substrates. Neither cyanide nor azide at 0.001 M concentration caused appreciable inhibition of the enzyme.

Methylene blue served sluggishly as a hydrogen acceptor. During the aerobic oxidation of nitroethane for each mole of nitrite produced approximately one mole of oxygen was consumed and one mole of aldehyde produced. The content of the enzyme was found to be greatest in *Neurospora* cultured on nitrate. When ammonia served as the sole nitrogen source, the enzyme content was greatly diminished or absent.

Conversion of protoporphyrin to bile pigment. IRVING M. LONDON, MARTHA YAMASAKI AND GLORIA SABELLA (introduced by D. RITTENBERG). *Dept. of Medicine, Columbia Univ., College of Physicians and Surgeons, New York City.*

Previous studies have shown that a fraction of pile pigment in normal man is derived from sources other than the hemoglobin of mature circulating erythrocytes (*Federation Proc.* 7: 169, 1948). In pernicious anemia and congenital porphyria, this fraction is increased (*J. Biol. Chem.* 184: 351, 359, 365, 1950). The identity of the additional sources is being investigated. It has been shown that hematin can be converted to bile pigment in the normal dog (*J. Biol. Chem.* 184: 373, 1950). This report is concerned with the utilization of protoporphyrin for bile pigment formation. Free protoporphyrin labeled with C^{14} was prepared from the hemoglobin of duck erythrocytes which had been incubated with $CH_3C^{14}OOH$; the labeled protoporphyrin had a C^{14} concentration of 3380 counts per minute. The protoporphyrin was dissolved in weak alkali and brought to pH 7.4. A total of 546 mg. of protoporphyrin in isotonic solution was given by slow intravenous infusion in divided doses on three consecutive days to a dog weighing 22.7 kg. Stercobilin was isolated in crystalline form from the feces and urine which were collected for eight days following the start of the experiment. The C^{14} activity of the stercobilin was 234 counts per minute, and the activity remained constant on recrystallization. These findings indicate that protoporphyrin can be converted to bile pigment in the mammalian organism. It remains to be determined whether this conversion is direct or whether it occurs via heme or some other intermediate.

Paper chromatography studies with *Lactobacillus bulgaricus* factors. CALVIN L. LONG, ROBERT A. MCRORIE AND WILLIAM L. WILLIAMS. (introduced by E. L. R. STOKSTAD). *Dept. of Biological Chemistry, North Carolina State College, Raleigh, N. C. and Lederle Labs. Div., American Cyanamid Company, Pearl River, N. Y.*

Earlier investigations established that the *Lactobacillus bulgaricus* factor (LBF) consists of at least 7 entities interchangeable for growth of the test organism. These different forms of LBF may be designated according to their position on a paper strip developed with ascending butanol. Inclusion of an inhibitory pantothenate analogue in the medium used for bioautography eliminated the areas of growth due to the most rapidly moving form (LBF-7), and to LBF-6 immediately below it. The growth zones due to LBF-1 (immobile) and LBF's 2 to 5 were relatively unaffected by the inhibitor. Omission of adenine from the medium resulted in the failure of certain LBF's to give growth areas, while other LBF's were unaffected. This may suggest the presence of adenine in the latter group. Omission of guanine similarly rendered some of the forms of LBF ineffective. One form of LBF which appears to be LBF-7 has been identified and synthesized by Snell and co-workers (*J. Am. Chem. Soc.* 72: 5349, 1950). Three forms of LBF arise from coenzyme A by digestion with intestinal phosphatase. Paper chromatography indicated that none of these were identical with LBF-7.

Response of serum glycine and alanine in rheumatoid arthritis after sodium benzoate. JOSEPH M. LOONEY, HENRY M. LEMON* AND WILLIAM H. CHASEN.* *Boston Regional Lab. of Veterans Administration and Boston Univ. School of Medicine, Boston, Mass.*

Rheumatoid arthritis is characterized by histologic changes of collagen. Because collagen contains 25% glycine, a study of the metabolism of this amino acid seemed advisable. The levels of the serum glycine and alanine, and the hippuric acid excretion were determined by the methods of Alexander and Weichselbaum, respectively, in 44 patients with rheumatoid arthritis and in 26 control subjects, of whom 14 had osteoarthritis, 7 had no evidence of bone disease, and 5 were ambulatory cancer patients. Determinations were made of hippuric acid one and two hours after intravenous administration of 1.77 gm. of sodium benzoate, and of glycine and alanine fasting and at one hour. There was no significant difference in hippuric acid excretion between the value of 0.87 + .03 mg. in the control group and that of 0.93 + .05 in the rheumatoid group. The latter gave 14 determinations less than 0.60 mg; the control group none. The fasting levels of glycine and alanine showed no significant differences. The values in mg. per cent were—for controls; glycine 2.77 ± 0.12, S.D. 0.59 ± 0.06; alanine 6.00 ± .41 S.D. 2.04 ± 0.28: for rheumatoid group; glycine 3.08 ± .11, S.D. 0.71 ± .07; alanine 5.70 ± .33, S.D. 2.17 ± .23. There was a significant difference in the percentage fall of glycine being three times as great in the rheumatoid group as in the control. The values were—for controls, 6.9 ± 1.3, S.D. 6.6 ± 0.9 for rheumatoid group 20.4 ± 2.3, S.D. 15.5 ± 1.6. The percentage fall in alanine showed

no significant difference, that for the controls being 14.9 ± 3.5, S.D. 17.9 ± 2.5; for the rheumatoid group 18.7 ± 3.2, S.D. 20.9 ± 2.2. All rheumatoid patients treated with ACTH or cortisone demonstrated a rise in serum alanine after sodium benzoate whereas previously they had shown decreases. Since almost all subjects showed normal liver function by other tests these results may indicate a defective peripheral glycine metabolism in rheumatoid arthritis.

Lipotropic properties of vitamin B_{12}. C. C. Lucas, Jessie H. Ridout,* Jean M. Patterson* and C. H. Best. *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Ontario, Canada.*

The lipotropic properties of vitamin B_{12} have been assessed in weanling male rats of the Wistar strain. Effects upon rate of growth, deposition of liver lipids and development of renal lesions have been followed for periods up to 17 days. The interrelationships among choline chloride, vitamin B_{12} and folic acid have been examined by supplementing a hypolipotropic basal diet with each one separately, at several levels, and in various combinations. Effects of the vitamins on food intake were controlled by conducting both *ad libitum* and paired-feeding experiments. Vitamin B_{12} had no effect on the liver lipids in these experiments.

Further studies on liver desoxyribonucleoprotein. J. Murray Luck, Robert Brunish,* DeLorez Fairley,* Donald Kupke* and Alberta Rhein.* *Dept. of Chemistry, Stanford Univ., Stanford University, Calif.*

From the livers of adult male rats and adult rabbits desoxyribonucleoprotein (DNP) was prepared. Nuclei were isolated by use of a citric acid technique at pH 6. The protein was prepared from the thoroughly washed nuclei by differential salt extraction and precipitation by dilution. The product was non-fibrous, of low viscosity in high salt, and soluble in water (1.3% at pH 6.5; over 3% at pH 8.5). The dialyzed lyophilized DNP contained 55–60% of crude protein, 30–35% of nucleic acid, and 7–9% of free lipid. In the presence of sodium chloride or potassium chloride of increasing concentrations the protein showed a broad zone of minimal solubility between 0.05 and 0.35 M salt. With magnesium, calcium, and zinc chlorides similar zones of virtual insolubility exist which extend from 0.005 M to 0.05 M (0.125 M for $ZnCl_2$) salt. DNP and rat liver histone, prepared from DNP, have been compared in respect to sedimentation in the analytical ultracentrifuge and also in respect to amino acid composition. Purine and pyrimidine base analyses on DNP have also been made. A determination of various cations by flame photometry reveals the presence of sodium, potassium, and calcium as the major cations in rabbit liver DNP.

Effect of x-irradiation on iron of serum and tissues of rats. Stephan Ludewig and Alfred Chanutin. *Biochemical Lab., Med. School, Univ. of Virginia, Charlottesville, Va.*

The iron concentrations of the sera of rats exposed to single doses (100 r to 1500 r) of x-rays are appreciably increased during the first 24 hours. The values return to the control range on the second or third day and tend to increase at the end of the first week following irradiation. The iron contents of the liver, spleen and kidneys of control and irradiated (500 r) rats were determined. After irradiation the values for the livers increase steadily during the first week and are all above the control range during the succeeding week. Despite the marked involution of the spleen after irradiation, the iron contents increase on the second day and remain elevated for about 3 weeks. Slight increases in the iron contents of the kidneys are noted about 10 days after irradiation.

Isoelectric points of amino acids and proteins by ionography. H. J. McDonald, M. C. Urbin, E. P. Marbach and M. B. Williamson (introduced by D. A. MacFadyen). *Dept. of Biochemistry, Graduate School and Stritch School of Medicine, Loyola Univ., Chicago, Ill.*

A substance migrating on paper under the influence of an uni-directional electric field will, with suitable precautions, move linearly with respect to potential and time, if the ionic strength and pH of the saturating buffer solution are kept constant. This fact makes it feasible to use this technique (described in *Arch. Biochem.*, in press.) to determine the isoelectric points of amino acids and proteins. An electrical potential (18.5v/cm.) is applied across the ends of filter paper ribbon, saturated with a buffer solution, to which are applied micro-quantities of the substance under study. If the mobility of the substance is measured in buffers of constant ionic strength, but varying pH, and the curve plotted, the point where the 'mobility vs. pH' curve crosses the zero mobility axis corresponds to the isoelectric point of the migrant. The isoelectric points of histidine, glutamic acid, casein and bovine serum albumin have been determined and found to be in excellent agreement with values determined by classical methods. For the proteins, veronal buffers of 0.0029 ionic strength were used; for the amino acids, several buffers at 0.013 ionic strength were employed. The isoelectric points, as determined by ionography, have been found to be: histidine, 7.6; glutamic acid, 3.1; casein, 4.6; bovine serum albumin, 4.9.

Purification and properties of adenosine triphosphate-light emitting system. WILLIAM D. McELROY AND JANE COULOMBRE (introduced by E. V. McCOLLUM). *Dept. of Biology, Johns Hopkins Univ., Baltimore, Md.*

The luminescent system from fireflies (*Photinus pyralis*) which emits light upon the addition of adenosine triphosphate (ATP) has been purified 100 times by $(NH_4)_2SO_4$ fractionation and adsorption on and elution from calcium phosphate gel. The purified system has lost all ATPase and myokinase activity with the result that only the terminal phosphate of ATP will support light emission. It is not possible to demonstrate a disappearance of labile phosphate groups during the luminescent reaction although the light intensity declines after the addition of ATP. A second addition of ATP, however, restores the initial high light intensity. The labile phosphate remaining in the preparations after the light intensity has decreased to a low level appears to be ATP since it can be transferred to glucose through the hexokinase reaction. The addition of inorganic triphosphate or pyrophosphate after ATP will stimulate light emission which is similar to a second addition of ATP. The results suggest that when ATP is first added to the luminescent system a small percentage is used in the light-emitting reaction while the remainder is complexed so that it is available to only a limited extent for light production. Presumably the inorganic triphosphate displaces the ATP from the complex. Kinetic analysis indicates that Mg^{++} is essential for the complexing reaction as well as the light-emitting reaction. The energies of activation for the two processes are practically identical. It is suggested that the complexing reaction is important for the utilization of labile phosphate in ATP for light emission.

In vivo inhibition of blood and tissue cholinesterases by DFP. MADELEINE L. MacFARLAND AND BERNICE E. HINES. (introduced by L. I. PUGSLEY). *Defence Research Board, Ottawa, Ontario, Canada.*

The rate and extent of inhibition of several blood and tissue cholinesterases have been studied in groups of rats dosed with DFP by the subcutaneous route. Determinations of enzyme activity were performed manometrically using the specific substrates acetyl-β-methylcholine and benzoylcholine for measuring true and pseudo-cholinesterases respectively. It was observed that the activities of liver and plasma pseudo-cholinesterases reached minimum levels within the first hour following injection whereas the true cholinesterases of the erythrocytes, plasma and brain underwent irreversible inactivation somewhat more slowly. The recovery patterns of the true and pseudo-cholinesterases of the plasma were very similar; evidence of regeneration of these enzymes, and also of liver cholinesterase, was obtained as early as 7 hours after dosing. In groups of rats receiving various sub-lethal amounts of DFP and sacrificed 4 hours following administration of the drug, the percentage inhibitions of the true cholinesterases of the blood did not differ significantly from that of the brain enzyme. It is concluded from these experiments that DFP becomes evenly distributed in the blood and tissues before interaction with cholinesterase enzymes takes place to an appreciable extent; consequently, blood true cholinesterase activities reliably mirror the state of inhibition of vital tissue cholinesterases. Measurements of plasma pseudo-cholinesterase activity are of little value in this regard owing to the marked sensitivity of this enzyme to inhibition by DFP.

Respiratory studies on slices of adrenal glands from rats, hamsters and guinea pigs. RALPH W. McKEE AND JEROME K. WALKER.* *Dept. of Biological Chemistry, Harvard Med. School and Cancer Research Inst. of New England Deaconess Hosp., Boston, Mass.*

It was desired to compare the oxidative metabolism of the adrenal glands of *1*) laboratory animals which synthesize their own ascorbic acid (rat and hamster) and *2*) an animal which depends on dietary vitamin C (guinea pig). The effects of *1*) various ions in suspending media, *2*) substrates, *3*) oxygen tensions, *4*) chilling and moistening, and *5*) ACTH, ascorbic acid and cyanide on the oxygen uptake of adrenal slices have been studied. With careful attention to the preparation of the tissue slices and employing a phosphate buffered isotonic medium with glucose as the substrate in an atmosphere of 100% oxygen at 38°C, with the conventional Warburg technique, the maximum oxygen utilization was obtained. Under these conditions, the oxygen uptake (cu. mm. per 100 mg. wet weight) of adrenal slices of young rats was an average of 156, while for adult rats the oxygen consumption averaged 104. The values for adrenals of adult hamsters averaged 130. With either young or adult guinea pigs the oxygen uptake was only about 75. Slices of guinea pig adrenal consisting principally of capsular and cortical tissues gave average values of 90, while slices containing a large amount of medullary tissue were only about 45. Cyanide depressed the oxygen uptake 60 and 15% at concentrations of 10^{-3} and 10^{-4} M, respectively. The addition of ascorbic acid to guinea pig adrenal slices had no influence on the rate of oxygen consumption. The possible influence on the oxygen utilization by adrenal slices with the addition of ACTH both

in vitro and *in vivo*, and of ascorbic acid deficiency are being studied further with the guinea pig.

Carbon dioxide fixation in relation to growth of *Serratia marcescens*. DOROTHY J. MCLEAN, ELIZABETH F. PURDIE AND NANCY HAWLEY ROBINSON (introduced by G. C. BUTLER). *Atomic Energy Project, Chalk River, Ontario, Canada.*

Carbon dioxide fixation in the bacterium *S. marcescens* was studied using bicarbonate labeled with C^{14}. After correcting for the production of inactive CO_2 by the bacteria it was found that the rate of fixation per mg. bacterial nitrogen was approximately ten times higher when the cells were in a growing condition than when the nitrogen source was omitted and the cells were in a physiological resting state. The total amount of CO_2 fixed when 0.05 mg. of nitrogen was assimilated from NH_4Cl was on the average 9.5 cu. mm. when the organic carbon source was glucose, glycerol or pyruvate and 6 cu. mm. when the carbon source was malate or citrate. When the same amount of nitrogen was supplied as asparagine the amount of CO_2 fixed with glycerol as substrate was the same as with citrate and about the value characteristic of malate or citrate plus NH_4Cl. The results suggest that a considerable portion of the CO_2 fixed during growth is synthesized into certain of the amino acids.

Effects of drugs and methionine sulfoximine on acetylcholine synthesis by brain slices. HUGH MCLENNAN* AND K. A. C. ELLIOTT. *Montreal Neurological Inst., Montreal, Quebec, Canada.*

Previously it was reported that the synthesis of acetylcholine (ACh) by brain slices under optimal conditions (bicarbonate-buffered medium containing 1.3 mM Ca^{++} and 27 mM K^+, other salts and glucose), is accelerated by 5 mM Metrazol and inhibited by 0.5 mM pentobarbital or higher concentrations of Metrazol. It has now been found that picrotoxin (0.02 mM), Metrazol (2.5 mM), camphor (0.1 mM), and thujone (12 μg., emulsion, per ml.), accelerate synthesis 25–100% while concentrations 4 times greater, or higher, inhibit. The narcotic drugs in very low concentration, pentobarbital (0.01 mM), amytal (0.02 mM), and avertin (0.2 mM) also accelerate synthesis by the same amount but inhibit in higher concentrations. If both convulsant and narcotic drugs are applied together in the low concentrations in which each alone would cause acceleration, a marked inhibition occurs such as would only be obtained by about ten times higher concentrations of the single drugs. Similar drug effects on ACh synthesis have been obtained with supplemented cell-free brain extracts. Methionine sulfoximine, the toxic factor of 'agenized' flour, has effects similar to those of the other drugs. The product from DL-methionine accelerates maximally at 1 mM and inhibits 50% at 5 mM. The product from L-methionine accelerates and inhibits to the same extent at half these concentrations. (The methionine sulfoximines were kindly supplied by Dr. L. Reiner, Wallace and Tiernan Products, Inc.)

Metabolism of 8-azaguanine in mice and monkeys. H. GEORGE MANDEL,* EDWARD L. ALPEN* AND PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

Studies have been made to determine the metabolic pathway of radioactive 8-azaguanine in CAF-1 mice with sarcoma 37. The 8-azaguanine-4-C^{14} was prepared from $NaC^{14}N$ and purified by extraction with *n*-butanol, while the 2-C^{14} compound was obtained from the Southern Research Institute. The 8-azaguanine was dissolved in sodium carbonate solution and injected subcutaneously. Excretion experiments with the 4-C^{14} compound have demonstrated that it is eliminated rapidly, with only 3% of the initial radioactivity remaining in the animals after 12 hours. Urine was collected from mice injected with 8-azaguanine-4-C^{14}, and was subjected to a 24-plate countercurrent distribution in a 1-molar phosphate buffer—*n*-butanol system at pH 6.7. Two compounds were shown to be excreted, neither one having an ultraviolet absorption spectrum of distribution coefficient identical with that of azaguanine. The major component comprised approximately 80% of the excreted radioactivity. Both components showed the typical pyrimidine absorption at 265 mμ. Identical results were obtained after an injection of 8-azaguanine-2-C^{14}. In tumor bearing and normal mice the same metabolic pattern was found. The metabolites have been characterized. A comparison was made between the urinary metabolic picture of azaguanine in monkeys and mice.

Some virucidal agents and their chemical and pharmacological properties. GEORGE H. MANGUN, A. R. KELLY,* B. E. SANDERS,* S. L. PIEPES,* A. M. WALLBANK* AND F. W. HARTMAN.* *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

Among 140 compounds screened, members of the following classes of compounds, listed in the order of their initial toxicity, are effective virucides in the presence of blood plasma: nitrogen and sulfur mustards (*I.* methyl-bis (2-chloroethyl)amine); imines (*II.* benzylethylenimine); conjugated unsaturated ketones (*III.* diacetylethylene); alkyl oxides (*IV.* ethylene oxide); acetophenones (*V.* chloroacetophenone); alkyl sulfates (*VI.* dimethyl sulfate); lactones (*VII.* beta-

propiolactone). While nearly as active a virucide as *I*, *VII* is less than 1/100th as toxic initially. Polyfunctional mustards and imines form protein addition products with reactive groups rendered more stable than molecules undergoing half hydrolysis with water. *IV* and *VII* partially react with chloride in plasma to form stable ethylene chlorohydrin (mouse LD_{50} 45 mg/kg. i.p.) and chloropropionic acid (mouse LD_{50} 1900 mg/kg. i.v.), respectively. *III* and *V* are much less effective virucides in whole blood than in plasma. It has been shown that cysteine reacts rapidly with these compounds, suggesting glutathione as one of the competing substances. All of the above compounds combine to some degree with the plasma proteins. Evidence for such reactions include low recoveries of hydrolysis products, more rapid disappearance from protein solutions, alterations in the proteins, and the direct demonstration of protein bound alkylating activity. Traces of undecomposed *I* and *II* may be neutralized with sodium thiosulfate, and *III* and *V* with sulfhydryl compounds.

Glycoaldehyde and glycolaldehydephosphate as reaction components in enzymatic pentose formation. J. MARMUR* AND F. SCHLENK. *Dept. of Bacteriology, Iowa State College, Ames.*

Triosephosphate can be combined with glycolaldehydephosphate to pentosephosphate by cell-free, purified bacterial enzymes from *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*. Glycolaldehyde instead of glycolaldehydephosphate gives a similar result. This condensation appears to be an important step in the formation of ribose nucleosides. Conversely, ribose-5-phosphate is split rapidly by our enzyme preparations under anaerobic conditions. This excludes breakdown by oxidative decarboxylation which is observed in other enzyme systems. Modified procedures and some new methods for the preparation and estimation of the compounds mentioned above have been developed. The relative significance of aerobic and anaerobic ribose metabolism will be discussed.

In vivo appearance of C-14 of DL-methionine-2-C-14 in fumarate, succinate, lactate and aconitate. LAWRENCE M. MARSHALL AND FELIX FRIEDBERG (introduced by VERNON A. WILKERSON). *Dept. of Biochemistry, College of Medicine, Howard Univ., Washington, D. C.*

Small amounts of C-14 appeared in fumarate, succinate, lactate and aconitate when mice were injected intraperitoneally with DL-methionine-2-C-14. Four, 7 and 10 hours after the administration of the labeled amino acid the animals were sacrificed and the livers were removed. After the addition of milligram quantities of fumaric, succinic, and aconitic acids, the extracts of liver were chromatographed on silica gel columns with a chloroform-butanol solvent. By modifying Marvel's procedure (*J. Am. Chem. Soc.* 72: 2642, 1950), the solvent was delivered to the column in such a way that the butanol component of the mixture progressively increased during the separation and thereby the heights of the chromatographic peaks were increased with a minimum loss of resolution for the particular system. The chemical nature of the effluent was determined spectrophotometrically. Average activities, expressed as counts per gm. liver per million counts of administered dose, were 21.2 and 8.2 for fumarate and aconitate, respectively. The value for combined succinate-lactate was 32.2. The degree to which radioactivity followed titratable acidity within the combined succinate-lactate zone was ascertained. Preliminary degradation studies indicated that at least a portion of the activity assigned to the combined succinate-lactate zone occurred in one or both of the non-carboxyl carbons of lactate.

Intestinal flora and cholesterol metabolism. WALTER MARX AND ELSIE WAINFAN.* *Dept. of Biochemistry and Nutrition, Univ. of Southern California, Los Angeles.*

Mice were fed a high cholesterol diet, and the total balance of cholesterol was determined. In agreement with previous reports, it was observed that approximately 15% of the cholesterol put in was either destroyed or so modified that it was not recovered when determined by a modified Schoenheimer-Sperry procedure. When, however, 1% sulfasuxidine and 0.04% streptomycin were added to the diet in order to decrease the activity of the intestinal flora, the total cholesterol put in was recovered, within the limits of error of the method. These results might be interpreted to indicate that, in the first group, the microorganisms in the intestine were primarily responsible for the destruction or modification of cholesterol. (Supported by U. S. Public Health Service.)

Studies of tryptophan metabolism in vitro. MERLE MASON* AND CLARENCE P. BERG. *Dept. of Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City.*

The fluorescence of a number of tryptophan intermediates has provided a convenient means for their detection after separation from biological media by paper chromatography. In addition to results previously reported (MASON, M. AND C. P. BERG. *J. Biol. Chem.* In press), this technique has yielded data concerning the metabolism of D-tryptophan and the role of vitamin B_6 in tryptophan metabolism. Rat liver slices caused the disappearance of D-tryptophan and D-kynurenine at very slow rates. Ammonia, keto acid and fluores-

cent intermediates were formed in only minute amounts, if at all. Kidney slices formed no fluorescent intermediates from D-tryptophan, but converted it fairly rapidly to ammonia and keto acid. They produced kynurenic acid and other fluorescent materials from D-kynurenine, though not as rapidly as from L-kynurenine. When small amounts of DL-kynurenine sulfate were administered to rats, a considerable portion of the D-component, but none of the L-isomer, was excreted. This observation was made possible by the separability of D-kynurenine from L-kynurenine on paper chromatograms. Homogenates of rat liver and kidney produced kynurenic acid and anthranilic acid from L-kynurenine. The role of pyridoxal phosphate in the formation of anthranilic acid from kynurenine (*Biokhimiya* 14: 163, 1949) was confirmed and was indicated also in the production of other substances. Chromatographic evidence showed that kynurenic acid formation was retarded in pyridoxine deficient tissues, but restored by the addition of pyridoxal phosphate.

Maximum rate of acetylation of para-aminobenzoic acid (= 'Lm-acetyl-PABA') by the liver of the rabbit. MORTON F. MASON, GUS CASTEN* AND ALLAN LINDSAY.* *Parkland Hosp. and Dept. of Internal Medicine, Southwestern Med. School of Univ. of Texas, Dallas.*

When the plasma level of PABA was sufficiently raised, in rabbits, by intermittent administration of the drug by stomach tube, the quantity acetylated per unit time increased to a maximum which was then independent of further increase in plasma concentration of the free drug. This maximum rate for a liver function (Lm) is analogous to a Tm function for the kidney. The acetylaminobenzoate formed was calculated by correcting the quantity excreted in urine (collected by catheter) for a given period by that amount accumulating in the body meanwhile. The latter was estimated, as an approximation, from the change in plasma concentration of acetyl-PABA during the period, assuming a previously determined average apparent volume of distribution equal to 24% of body weight. Lm-acetyl-PABA, reasonably constant in an individual, exhibited considerable variation in a series of 10 male rabbits, ranging from 0.39–0.89 mg. (expressed as equivalent of free drug) per minute per kg. body weight. Administration of sodium acetate with PABA (3 experiments) did not increase Lm-acetyl-PABA, indicating acetate availability is not a limiting factor determining the maximum rate under these conditions.

Investigations on golden nematode hatching factor. LOUIS M. MASSEY, JR. AND A. LESLIE NEAL (introduced by HAROLD H. WILLIAMS). *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*

The hatching of the Golden nematode (*Heterodera rostochiensis* Wollenweber) or potato eelworm is known to be dependent upon an unknown organic substance excreted by the roots of potato and tomato plants. Aqueous leachings from the potted plants contain this hatching factor for which an assay procedure has been developed. Concentration of the active substance has been obtained without appreciable loss of activity by: *1*) lyophilizing leachings from potted tomato plants; *2*) extracting the resulting residue with absolute ethanol; *3*) concentration of the ethanolic solution under vacuum; and, *4*) partial precipitation of impurities by the addition of ether. Subsequent purification has been accomplished by employing paper chromatographic techniques using ascending development with 80% aqueous phenol. The R. F. value for the factor has been found to be between 0.83 and 0.88. The hatching factor has been shown to be present in tissue homogenates of tomato leaves and roots and in leaf culture media. A hatching inhibitor has been demonstrated to be present in leachings obtained from two-week old tomato plants growing in steam sterilized soil. Concentrates of these leachings when added to solutions containing sufficient hatching factor to produce high activity were found to completely inhibit the hatching of nematode larvae.

Nature of alterations in activity of alkaline phosphatase in tissues of hypophysectomized rats. JAMES C. MATHIES (introduced by O. H. GAEBLER). *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

Previous findings of decreased alkaline phosphatase activity in bone and kidney and increased activity in liver following hypophysectomy, with returns toward normal control values on treatment with large amounts of anterior pituitary growth hormone preparations (*Endocrinology* 45: 129, 480, 1949), are being investigated in greater detail to aid in assessing the physiological importance and meaning of these findings. Treatment with 'physiological' amounts (small doses) of growth hormone has now been shown to be as effective in restoring renal and tibial alkaline phosphatase activities after hypophysectomy, as the large amounts used previously, although liver alkaline phosphatase was unchanged or further increased under these conditions. Activities observed in animals 6 months postoperatively indicate that the effects of the operation on tibial, renal and hepatic alkaline phosphatase persist long after the glandular ablation. The enzymic response to growth hormone treatment 6 months after hypophysectomy will also be reported. Treatment of operated animals with thyroxine

resulted in no change in body weight, food intake or hepatic alkaline phosphatase, while reducing renal and considerably increasing tibial alkaline phosphatase. By contrast, in intact animals the same treatment resulted in precipitous weight loss, reduction in hepatic and renal alkaline phosphatase activities and very slight increases in tibial phosphatase. Data will be presented showing that these results are independent of the manner of feeding of the animals and the use of magnesium activation in the enzyme estimation.

Determination of serum iron. JOHN W. MEHL AND JACQUELINE GERJUOY.* *Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Southern California, Los Angeles.*

The serum iron method proposed by Burch, Lowry, Bessey, and Berson (*J. Biol. Chem.* 174: 791, 1948) offers certain advantages with respect to simplicity. However, we have not found the correction for hematin to be adequate when hemolysis is great. A more serious difficulty arises in the presence of jaundice, since bilirubin is extracted into the isoamyl alcohol, and interferes in the determination. In view of their report that recovery of iron in trichloracetic acid filtrates of serum is incomplete, we have re-investigated this point. A comparison with the o-phenanthroline procedure and some applications will be discussed. (Contribution #279 from the department of Biochemistry and Nutrition.)

Mechanism of oxidations catalyzed by chloroplasts. ALAN H. MEHLER AND ALLAN H. BROWN (introduced by H. GAFFRON). *Inst. of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill., and Dept. of Botany, Univ. of Minnesota, Minneapolis.*

In the course of studies on reactions catalyzed by illuminated isolated chloroplasts, a formation of hydrogen peroxide was detected by using catalase as a peroxidase for the oxidation of ethanol. A mechanism has been proposed involving reduction of oxygen by hydrogen liberated from water with simultaneous evolution of oxygen from the remaining fragment of the water molecule. A catalytic effect of manganous ions on this reaction has been found. Ascorbic acid and glutathione are oxidized only slowly in the presence of isolated chloroplasts. If chloroplasts are first allowed to reduce quinone in the light, they are then capable of oxidizing ascorbic acid or glutathione at rates faster than any other known reactions of chloroplasts. Hydroquinone has no effect on this reaction. This oxidation requires no supplementary enzymes and proceeds only in light. The mechanism by which molecular oxygen is evolved and consumed was studied with use of O^{18} enriched gas. Analyses were performed with a semi-continuous automatic recording mass spectrometer. Changes in the mass peaks 32 ($O^{16}O^{16}$) and 34 ($O^{18}O^{16}$) to be discussed lend partial support to the mechanism proposed. These studies also demonstrate that, in the absence of other Hill oxidants or peroxide trapping agents, illuminated chloroplasts catalyze an exchange between the oxygen of water and gaseous oxygen. This exchange is in agreement with the proposed mechanism for oxygen consumption.

Studies on isoleucine and its keto analogues. ALTON MEISTER. *Natl. Insts. of Health, National Cancer Inst., Bethesda, Md.*

Enzyme preparations obtained from hog heart muscle and Lactobacillus arabinosus were found to catalyze the formation of L-glutamic acid from α-ketoglutaric acid and L-isoleucine or L-alloisoleucine. Deamination of these amino acids to ammonia did not occur, nor was glutamate formed from α-ketoglutarate and ammonia under these conditions. Glutamate formation from each of the isoleucine isomers was accelerated by pyridoxal phosphate. The bacterial preparation also catalyzed L-glutamic acid formation from a number of other L-amino acids (excluding allothreonine, threonine, and alanine) but was not active with the D-isomers of these amino acids or of isoleucine and alloisoleucine. The keto analogues of isoleucine were isolated as the crystalline sodium salts after enzymatic oxidation of the corresponding D-amino acid isomers. The formation of isoleucine from *d*- and *l*-α-keto-β-methylvaleric acids in the presence of pyridoxal phosphate and L-glutamate was demonstrated with the heart muscle and bacterial preparations. However, the transamination reactions proceeded more readily in the direction of glutamate formation. Although racemization of α-keto-β-methylvaleric acid occurred rapidly in alkaline solution, neither keto acid isomer exhibited significant loss of optical activity when incubated at 37° for 15 hours at values of pH less than 8.4. Both *d*- and *l*-α-keto-β-methylvaleric acids, as well as L-alloisoleucine supported the growth of Lactobacillus arabinosus cells on a medium deficient in L-isoleucine. The possibility that a biological mechanism exists for the racemization of these keto acids will be discussed.

Hydrolysis of glucuronidic linkage of hyaluronic acid. KARL MEYER AND ALFRED LINKER.* *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hosp., New York City.*

Purified testicular hyaluronidase hydrolyzes only the N-acetylglucosaminidic bonds of hyaluronic acid. At completion of the hydrolysis,

di- and tetrasaccharides with the free reducing group in the glucosamine moiety are found. (M. M. RAPPORT, K. MEYER AND A. LINKER. *J. Am. Chem. Soc.* In press). Incubation of the hydrolysis mixture with a crude testicular fraction yields glucuronic acid and N-acetylglucosamine as shown by analysis and paper chromatography of effluent fractions of the carbon column used. The enzyme liberating the monosaccharides is a β-glucuronidase which can be separated by one $(NH_4)_2SO_4$ fractionation from hyaluronidase. The crude testicular fraction likewise hydrolyzes borneol and phenolphthalein β-glucuronides, but not β-glucuronidogalactose (M. HEIDELBERGER AND F. E. KENDALL, *J. Biol. Chem.* 84: 639, 1929) or the disaccharide formed by pneumococcal hyaluronidase from hyaluronic acid. Purified liver glucuronidase hydrolyzes the same fractions as the crude testicular enzyme. The specificity of the β-glucuronidases is being investigated. (This work was supported by the Helen Hay Whitney Foundation.)

Stability toward heat of enzymes obtained from a thermophilic bacterium. WALTER MILITZER AND L. CONSTANCE TUTTLE (introduced by S. B. BINKLEY). *Dept. of Chemistry, Univ. of Nebraska, Lincoln.*

In studies on a thermophilic bacterium (isolate #2184) we have shown that the property of stability toward heat accompanies the enzymes of a cell particle released from the bacterium (*Arch. Biochem.* 24: 75, 1949; 26: 299, 1950). One of these enzymes, adenosinetriphosphatase, released from the insoluble particle by acetone treatment has been purified sufficiently by ammonium sulfate precipitation so that its activity could be studied in dilute solution (0.5 mg. per ml. and less). From the results in dilute solutions it does, indeed, seem that the stability toward heat (65°C., 120 minutes, pH 6.8) is an inherent property of the adenosinetriphosphatase itself and that it is not due to some protective ion or molecule.

Mechanism of plasma protein synthesis by the liver as studied with the aid of Lysine-ε-C^{14}. LEON L. MILLER AND CHAUNCEY G. BLY.* *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Plasma protein synthesis has been studied *in vitro* by the perfusion technique previously described (*Federation Proc.* 9: 206, 1950). In experiments in which liver proteins are labeled with lysine-ε-C^{14} the administration of unlabeled amino acids fails to release labeled proteins from the liver in significant amounts. The distribution of the radioactivity among the formed elements of the labeled liver will be presented, and a working hypothesis for the synthesis of plasma proteins will be presented. An essential feature of this hypothesis is the demonstration of the absence of an obligate intracellular precursor of the circulating plasma proteins.

Phosphoglyceric acid utilization by water extracts of cardiac muscle. O. NEAL MILLER (introduced by WENDELL H. GRIFFITH). *Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Branch, Galveston.*

Experiments with cardiac muscle slices reported previously, (*Federation Proc.* 9: 207, 1950) have indicated that metabolic pathways of pyruvate and lactate may converge toward phosphoglyceric acid, but by different pathways. In this investigation, the utilization of phosphoglyceric acid has been determined and an attempt made to follow the fate of phosphoglyceric acid-carbon. Calf hearts were taken from the slaughter house within a few minutes after death of the animal, chilled, and an acetone powder prepared (from the ventricle) in the usual manner. Acetone powder was ground in a mortar with potassium phosphate buffer, (pH 7.4), centrifuged and experiments performed on the supernatant fluid. The extract was incubated in the standard Warburg vessel at 37° under an atmosphere of air for one hour. Included in the reaction mixture were, magnesium and sodium ions, phosphoglycerate, creatine, muscle adenylic acid and DPN. In addition, some flasks contained varying concentrations of fluoride. Phosphoglycerate disappearance was determined and the flask contents were titrated with hypoiodide and also analyzed for lactate and pyruvate by conventional methods. It has been observed that a concentration of sodium fluoride 10 times higher than is known to completely inhibit enolase, failed to inhibit, completely, the utilization of phosphoglyceric acid. The percentage inhibition was never more than 70%. The formation of lactate and pyruvate failed to account for disappearing phosphoglyceric acid. The significance of these results will be discussed.

Enzymatic disappearance of formaldehyde in the presence of pyruvate. PEGGY MITCHELL* AND CAMILLO ARTOM. *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*

Formaldehyde, incubated with homogenates of most animal tissues, is partly converted into compounds not detectable with chromotopic acid. The addition of pyruvate greatly enhances the rate of disappearance of formaldehyde. A similar, but less marked effect is obtained with alpha-ketoglutarate and oxaloacetate. From various lines of evidence (formation of the 2,4-dinitrophenylhydrazine derivative, oxidation with periodate, isotope determinations after incubation with $HC^{14}HO$) it appears that the reaction might be: $CH_3 \cdot CO \cdot COOH$

+ $H \cdot CHO \rightarrow CH_3 \cdot CO \cdot CH_2OH + CO_2$. The enzyme responsible for this reaction is stable at 37° and is completely inactivated only at relatively high temperatures (one hour at 60°–65°). It is resistant to concentrations of iodoacetate which inhibit the disappearance of formaldehyde in the absence of pyruvate. The enzyme is present in the large granule fraction ('mitochondria') of rat liver, little, or no formaldehyde being removed by the granules without added pyruvate. Our preparations were not activated by Mg^{++}, thiamine or cocarboxylase. However, the differentiation of the formaldehyde-pyruvate condensing enzyme from the diphosphothiamine-metal-proteins catalyzing the formation of acyloins from other aldehydes and pyruvate (GREEN *et al. J. Biol. Chem.* 145: 69, 1942) remains uncertain. (Work performed under Contract AT-(40-1)-288, Title II, with the U. S. Atomic Energy Commission).

Precursors of beta carbon of serine and of methionine methyl group. CHOZO MITOMA* AND DAVID M. GREENBERG. *Biochemistry Div., Univ. of California School of Medicine, Berkeley.*

The conversion of portions of certain compounds labeled with C^{14} to the beta carbon of serine and the methyl group of methionine was studied in preparations from rat liver of concentrated homogenates and suspensions of mitochondria in phosphate buffered media. Representative results of the percentage conversion of the label aerobically in homogenates to serine are: sarcosine-CH_3-C^{14}, 60; glycine-2-C^{14}, 3.0; betaine-CH_3-C^{14}, 0.2; formaldehyde, 0.36; formate, 0.12. For methionine methyl formation representative results with the same labeling are: betaine, 3.0; sarcosine, 1.0; glycine, 1.0; formaldehyde, 3.0; formate, 0.05. The conversion of formaldehyde and sarcosine to serine was greater with mitochondria than unfractionated homogenates. Inert glycine increased and inert formaldehyde reduced the conversion of the methyl groups of sarcosine to serine. The formaldehyde to serine conversion goes better anaerobically. The radioactive products of the incubations can be separated chromatographically with a Dowex-50 column by HCl elution.

Effect of lysine on metabolism of minced one-day-old mouse brain and propagation of Theiler's GD VII virus. K. MOLDAVE,* RICHARD J. WINZLER AND HAROLD E. PEARSON.* *Depts. of Biochemistry and Microbiology, Univ. of Southern California School of Medicine, and Lab. Div., Los Angeles County Hosp., Los Angeles, Calif.*

The propagation of Theiler's virus *in vitro* in a media containing minced one-day-old mouse brain was found to be inhibited by lysine. This inhibition could be partially reversed with methionine. Investigations using C-14 uniformly labeled glucose, C-14 carboxyl labeled lysine, and C-14 methyl labeled methionine have been carried out in an effort to elucidate the mechanism of lysine inhibition. It has previously been shown that the virus stimulates the uptake of C-14 from C-14 glucose into the proteins of minced one-day-old mouse brain cultures while inhibiting the uptake into the lipids. In the presence of lysine, the incorporation of radioactivity into the proteins and the lipids is markedly reduced in both the virus infected and in the noninfected cultures. Methionine alone has no effect on this process, but in the presence of lysine, it partially reverses the decreased incorporation of radioactivity into the lipids and the proteins of both infected and noninfected cultures. Theiler's virus also stimulates the uptake of radioactivity from C-14 methyl labeled methionine into the proteins while inhibiting the incorporation into the lipids. The effects of lysine on the uptake of C-14 from labeled methionine as well as the incorporation of C-14 from labeled lysine have been investigated. (Contribution number 282 from the Department of Biochemistry and Nutrition.)

Role of adenosine triphosphate in molecular transformations of actin. W. F. H. M. MOMMAERTS (introduced by P. HANDLER). *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.*

Actin is known to occur in solution in two forms, globular (G-) and fibrous (F-) actin. The latter arises from the former by polymerization, elicited by neutral salts in the presence of adenosine triphosphate (ATP). Polymerized actin can be separated from its monomer and from other globular proteins by preparative ultracentrifugation. It can be depolymerized reversibly in the presence of ATP. In the transformation of G- to F-actin, ATP is incorporated into, or bound by the polymerizing protein. After ultracentrifugal sedimentation of the fibrous protein, a considerable fraction of the nucleotide remains in the supernatant. A definite amount, however, is held by the protein. This can be determined after extraction of the pellet, making due correction for the solvent enclosed in the pellet by determining the weight and protein content of the latter. The results of such determinations, under varying conditions of pH and ATP concentration, indicate a stoichiometric combination between actin and the nucleotide. The velocity of polymerization is, within certain limits, enhanced by increased amounts of ATP, but the quantity of ATP incorporated by the polymerizing protein does not rise under those conditions. Although the amount of actin in muscle is not accurately known, it may be estimated that the amount of ATP bound by

the actin of e.g. one gm. of muscle is about the same as the quantity of ATP in this amount of muscle which is decomposed in one single twitch. It is suggested, therefore, that the reaction between actin and ATP is involved in the fundamental process of contraction.

Oxidation of tricarboxylic acid cycle intermediates by lactating mammary gland cyclophorase. RICHARD O. MOORE AND WALTER L. NELSON (introduced by JAMES B. SUMNER). *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*

Cyclophorase has been prepared from the mammary gland of lactating guinea pigs essentially by the method described by Green, Loomis and Auerbach (*J. Biol. Chem.* 172: 389, 1948) for kidney and liver. Such preparations differ in important respects from the cyclophorase of kidney. When prepared fresh, the enzyme system will not oxidize pyruvate or any of the citric acid cycle intermediates in the absence of added adenosine triphosphate (or adenylic acid) and magnesium ions. When ATP and Mg^{++} are supplemented, the mammary gland cyclophorase readily oxidizes α-ketoglutarate and succinate, while fumarate is oxidized only very slightly, and citrate and malate not at all. Stechiometric data indicate the α-ketoglutarate and succinate are oxidized only to the extent of fumarate under these conditions. In agreement with the work of Green *et al.* on kidney, the concurrent addition of pyruvate with fumarate results in rapid oxygen consumption. If coenzyme I and glutamate are added to the system containing malate, the malate is now readily oxidized. Likewise if the system containing citrate is supplemented with coenzyme II, the citrate is oxidized. These data would tend to indicate that the bound pyridine nucleotides which have been demonstrated for kidney and liver (HUENNEKENS AND GREEN, *Arch. Biochem.* 27: 418, 1950) are absent from freshly prepared cyclophorase of mammary gland tissue.

Cholesterol metabolism in pathogenic acid deficiency. AGNES FAY MORGAN AND RAYMOND R. GUEHRING.* *Dept. of Home Economics, Univ. of California, Berkeley.*

Pantothenic acid-deficient rats fed a diet containing 1% cholesterol did not develop fatty or cholesterol-rich livers but had moderately raised blood cholesterol levels. Rats on normal diets plus cholesterol developed fatty livers in 7–14 days but on withdrawal of pantothenic acid marked reduction in liver fat and cholesterol, and in blood cholesterol occurred in 3 to 6 weeks. Neither pyridoxine nor riboflavin-deficient animals failed to produce fatty livers when fed the cholesterol-rich diet. The food intakes of normal and deficient rats were equalized. Pantothenic acid-deficient dogs on the contrary developed fatty livers both with and without addition of 1% cholesterol to the diet but maintained normal liver cholesterol and reduced blood cholesterol levels. The cholesterol-fed deficient dogs had normal adrenals but the other deficient dogs had hemorrhagic adrenals. Dogs fed a normal diet plus 1% cholesterol had fatty and cholesterol rich livers and raised blood levels of cholesterol. Liver biopsy followed by doubling of the choline ration showed reduction of liver fat and cholesterol to normal in 3–5 months but only partial reduction of liver fat followed withdrawal of pantothenic acid. The synthesis of cholesterol appears to be dependent to some extent on pantothenic acid, perhaps in coenzyme A, so that exogenous cholesterol is well tolerated by pantothenic acid-deficient rats and dogs.

Types of liver glycogen. MARTIN MORRISON,* ADRIAN C. KUYPER AND JAMES M. ORTEN. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

The types of glycogen deposited in the liver of the rat in various experimental conditions including fasting and following the administration of glucose and galactose are being investigated. Since the method of isolation may affect its structure, glycogen was isolated by both aqueous extraction and modified Pflüger's procedures. The various preparations were studied by adsorption analysis, by end group determinations, and by iodine absorption spectra. In adsorption analysis, a dilute aqueous solution of the glycogen preparation was passed through a column of tricalcium phosphate and fractions were collected with a fraction collector. The concentrations of glycogen present in the various fractions were determined quantitatively by the anthrone method. When the volume of the solution which had passed through the column was plotted against the concentration, typical frontal analysis curves (S. CLAESSON, *Arkiv für Kemi.* 26A: no. 24, 1949) were obtained. This procedure provides information as to the homogeneity and structure of the polysaccharide. The glycogen fractions were further characterized by the end-group determinations based on periodate oxidation and by the absorption spectra of the iodine-treated preparations.

Degradation of alpha-ketoglutaric, glutamic and citric acids. E. H. MOSBACH, E. F. PHARES AND M. V. LONG (introduced by C. G. KING). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Isotopic tracer studies of the participation of alpha-ketoglutaric, glutamic and citric acids in various biological systems (now being carried on

in this and other laboratories) have demonstrated the need for complete and convenient degradation procedures for these acids. The known degradation methods usually do not permit a unique determination of the isotope concentrations of each separate position of the carbon skeleton and result in the determination of average values only for two or more carbon atoms. A new degradation procedure will be described which permits the measurement of the isotope concentration (specific activity) of each carbon atom of alpha-ketoglutaric, glutamic and citric acids. The following series of reactions was used: citric acid → alpha-ketoglutaric acid → glutamic acid → succinic acid semialdehyde → butyric acid → propionic acid. The propionic acid was then degraded further by successive Schmidt reactions. This scheme therefore also affords a convenient stepwise degradation of butyric acid. Experimental details, proof of the procedure with compounds of known isotopic composition, and biochemical applications will be discussed.

Heat inactivation of salivary amylase. JYTTE MUUS. *Dept. of Physiology, Mount Holyoke College, South Hadley, Mass.*

Crystalline salivary amylase was prepared from pooled human saliva by fractionation with acetone and salts. Three times recrystallized amylase was used in the experiments. The rate of heat inactivation of the enzyme in buffers and in the presence of different preparations was determined. When previously denatured amylase was added to the native enzyme the rate of destruction was greatly decreased. Proteins such as crystalline ovalbumin had much less effect. The protective action of the denatured enzyme was also indicated by the decrease in rate of reaction during inactivation. Some type of interaction between native and denatured enzyme rather than reactivation of the denatured amylase seems to account for the results. Boiled saliva and denatured partially purified amylase stabilized the crystalline amylase considerably more than could be accounted for on the basis of their amylase content. This would indicate that saliva contains some other substance which stabilizes the amylase and which is carried through part of the purification process. In some cases it has been found extremely difficult to rid the crystalline enzyme of stabilizer.

Crystallization and properties of 'direct' and 'indirect' bilirubin from human serum. VICTOR A. NAJJAR. *Dept. of Pediatrics, Johns Hopkins Univ., Baltimore, Md.*

Essentially the same procedure was used for both bilirubins. For the 'direct,' serum was obtained from cases with occlusion of the bile ducts, and for the 'indirect' from cases with hemolytic jaundice, congenital bilirubinemia, jaundice of the newborn and from hematoma fluid. One ml. or more was used. The serum was brought down to pH 6.0 by 0.2 M phosphate buffer pH 6.0 and extracted with ether, washed with water and extracted with 0.01 M pyrophosphate buffer pH 9.5. The 'indirect' was recovered almost quantitatively; however, only a fraction of the 'direct' was recovered because of its poor solubility in ether. The pyrophosphate extracts of both bilirubins contained no detectable protein and showed identical absorption maxima at 440 mμ; nevertheless, the bilirubins still retained their original 'direct' and 'indirect' van den Borgh reactivity. The 'indirect' extract was made 'direct' reacting by mild treatment with weak alkali at pH 11. For crystallization of the 'indirect' the pH of the extract was brought down dropwise to 7.6 with 0.2 M phosphate buffer pH 7.0 and cooled in the icebox. In one hour crystal formation began and was complete overnight. The 'direct' was more difficult. The pH was lowered to 7.2 very slowly over many hours and was left 24 hours in the icebox followed by gradual freezing. Crystals from 'indirect' extracts were mostly fine long needles sometimes arranged in rosettes. Occasional rhomboid plates appeared. From the 'direct' extracts, crystals were thick short, rod shaped with blunt ends.

Production of antibody to Welchii antitoxin that inhibits lecithinase activity of the corresponding toxin. VICTOR A. NAJJAR. *Dept. of Pediatrics, Johns Hopkins Univ., Baltimore, Md.*

Zamecnik and Lipmann (*J. Exper. Med.* 85: 395, 47) showed that Welchii antitoxin is directed towards the enzymatic center of the toxin (lecithinase). An antibody formed against the reactive surface of the antitoxin might resemble the enzymatic center of the toxin sufficiently to inhibit its activity by competing for the substrate. Five rabbits were injected subcutaneously with refined Welchii antitoxin (Lederle), 330 antitoxin U every 3 days for 6 doses. One rabbit gave a precipitin titer of 1:1,000,000. The others, 1:10,000 or less. Serum from that rabbit consistently inhibited lecithinase activity 30% and 12% during the first and second hour of incubation, respectively, as compared to normal serum. It did not inhibit alcohol dehydrogenase, *d*- and *l*-amino acid oxidase obtained from liver extracts. The other sera and those obtained from 4 rabbits immunized with B. Coli showed no inhibition of (toxin) lecithinase activity. When a booster dose was given to that rabbit with inhibiting serum, a large area of necrosis developed at the site. Ten ml. reaction mixture contained Lecithin 0.5%, Calcium Chloride 0.005 M, Serum 1.0 ml., an amount of toxin to liberate no more than 200

phosphorus per hour, borate buffer 0.03 M pH 7.3. Temperature 30°C. The enzyme activity was followed by measuring liberation of phosphoryl choline as acid soluble phosphorus.

Properties and occurrence of a diphosphopyridine nucleotide-splitting enzyme from *Neurospora*. ALVIN NASON (introduced by NATHAN O. KAPLAN. *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

An enzyme from *Neurospora crassa* has been detected which hydrolytically cleaved DPN and TPN at the nicotinamide-riboside linkage. The enzyme has been purified 70 times from zinc deficient mats where it occurs at a 10-fold greater concentration than in normal tissues (NASON, KAPLAN AND COLOWICK. *J. Biol. Chem.* In press). One mg. of the purified DPNase splits 3000 μM of DPN per hour at 37°C. corresponding to a turnover number of about 5000. The enzyme has no clear-cut pH optimum and is active from pH 3–9. Neither addition of various metals nor heavy metal binding agents affected activity. The production of one mole of acid, the appearance of one reducing group, the disappearance of the nicotinamide-riboside bond, the appearance of free nicotinamide, and the isoltation of adenosine-diphosphate ribose as a reaction product indicated splitting to occur at the nicotinamide-riboside linkage. The enzyme is similar to brain DPNase in its specificity to a number of N-substituted pyridinium compounds, failing to attack nicotinamide mononucleotide and nucleoside, and half- and fully-reduced forms of DPN. Nicotinamide which markedly inhibits the animal enzyme has no effect on the *Neurospora* enzyme. The enzyme appears in elevated amounts (10-60-fold increase) not only in individual zinc and biotin deficiencies but also in nitrogen deficiency, simultaneously with the disappearance of several other enzymes tested. Conditions necessary for restoring the enzyme pattern of deficient mats to normal will be described.

Calculation of amount of sodium bicarbonate to be administered to patients in acidosis to bring to normal pH. SAMUEL NATELSON. *Dept. of Biochemistry, Rockford Memorial Hosp., Rockford, Ill.*

An equation is derived for calculating the amount of sodium bicarbonate to be administered intravenously to patients in acidosis to bring to normal pH as measured by the pH meter. mEq. of $NaHCO_3$ (M) to be administered per liter of extracellular fluid (per 5 kg. body weight) is calculated from the derived equation: $M = 26\ (19.95 - y)/y + 1$, where y = antilog (pH of the patient − 6.1). If the patient is brought to pH 7.4 and then urinates he will tend to go into alkalosis unless saline is administered in amounts designed to correct chloride deficiency.

Blood pH	7.00	7.10	7.20	7.30	7.40
M	34.8	23.3	14.0	6.22	0
$NaHCO_3$ (gm.)	2.92	1.96	1.18	0.52	0
Na lactate equiv. (ml.) (1/6 M)	209	140	84	38	0

This table has been successfully used in children and adults suffering from severe acidosis with anuria.

Role of phosphatase. W. F. NEUMAN, V. DI-STEFANO* AND B. J. MULRYAN.* *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.*

Working on the hypothesis that ester phosphate is inhibitory to calcification in the absence of phosphatase, experiments of three basic types were carried out. In the first type of experiment, studies on rachitic bone slices demonstrated that ester phosphate does indeed inhibit calcification when phosphatase is inactivated. In conjunction with this, further experiments were performed to demonstrate the inhibition of the accretion of mineral by ester phosphate in a purely physical system. In experiments of the second type, radioactive glycerol phosphate was synthesized and shown to be adsorbed by the bone mineral. The third type of experiment demonstrated the complexing ability of ester phosphate for calcium. This was accomplished by the addition of varying amounts of calcium to a solution containing glycerol phosphate and sulfate. The foregoing experiments indicate that phosphatase has a three-fold role in the calcification process: *1*) phosphatase increases the concentration of free inorganic phosphate as first suggested by Robison. *2*) Phosphatase increases the concentration of calcium by destroying ester phosphate, the complexing agent. *3*) Phosphatase exerts a protective action by preventing the adsorption of ester phosphate which is inhibitory to the growth of the mineral crystal. (Based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.)

Incorporation of radioacetate into 17-hydroxycorticosterone by adrenal cortex slices. E. D. NIELSON, N. A. DRAKE AND W. J. HAINES (introduced by M. H. KUIZENGA). *Research Labs., Upjohn Company, Kalamazoo, Mich.*

When the incorporation of radioactive carbon from acetate into adrenal cholesterol was demonstrated by Srere *et al.* (*J. Biol. Chem.* 176: 829, 1948) the question arose: would such carbon also enter the adrenal steroid molecule? Using essentially the same incubation conditions as in the above work, we have been able to show the in-

corporation of acetate carbon into 17-hydroxycorticosterone. A total of 30 gm. of fresh hog adrenal cortex slices were incubated for two hours at 37.5°C. in Krebs Ringer bicarbonate solution which contained ACTH and $CH_3C^{14}O_2Na$. One mc. of C^{14} was used. The incubated solution and slices were extracted with acetone and the neutral hormone concentrate was obtained by a standard procedure. The hormone concentrate was subjected to paper chromatography according to the technique of Zaffaroni *et al.* (*Science* 111: 6, 1950). Auto-radiograms indicated that there was radioactivity in the spot corresponding to 17-hydroxycorticosterone (Kendall's Compound F). Repeated chromatography in different systems failed to separate the radioactivity from the compound F spot. There was also no separation of the 17-hydroxycorticosterone and radioactivity when known compound F was added, and when derivatives of the material were paper chromatographed. Hog adrenal brei, incubated under the same conditions, gave no indications of having incorporated radioactivity into any of the steroid molecules. (The early parts of this investigation were done with the advice and assistance of Dr. Austin M. Brues and Dr. William P. Norris of the Argonne National Laboratory, Chicago, Illinois.)

Observations on respiration and phosphorylation with liver mitochondria of normal, hypo-, and hyperthyroid rats. HERMANN NIEMEYER,* ROBERT K. CRANE,* EUGENE P. KENNEDY* AND FRITZ LIPMANN. *Biochemical Research Lab., Massachusetts General Hosp., Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Mitochondria suspensions were prepared by following essentially the method of Hogeboom and Schneider. Hypothyroid preparations were obtained from animals after 2–3 weeks' interval from thyroidectomy. Hyperthyroid conditions were elicited by daily intraperitoneal injections of 1.5 mg. of thyroxin for 3–5 days. The QO_2 was measured with glutamate, magnesium, ATP, phosphate, and fluoride at optimal concentrations. Under such conditions, hyperthyroid preparations showed a 30–50% increase in respiration, while athyroid preparations did show a small, barely significant depression against normal. No significant difference in P/O ratios could be detected. It was found during these experiments that normal respiration is increased up to 50% by addition of a phosphate acceptor system, namely, hexokinase plus glucose. Controls with glucose-6-phosphate, hexokinase, or glucose alone were negative. This indicates a depression of the rate of hydrogen transfer by accumulation of phosphorylated intermediaries. With thyrotoxic preparations, this increase, due to the addition of hexokinase plus glucose was absent or diminished, while athyroid preparations showed a definite but relatively small effect. The ATP-ase activity of our hyperthyroid and normal liver preparations did not differ. It may thus be that in the thyrotoxic preparation, a less pronounced backing up of phosphorylated intermediaries occurs.

Kinetics of the transaminase reaction. ALFRED NISONOFF AND FREDERICK W. BARNES, JR., (introduced by W. M. CLARK). *Depts. of Medicine and Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Md.*

With the glutamic-aspartic transaminase system, a study of enzyme behavior was begun by a systematic investigation of the variables involved in kinetic studies, by precision measurements of initial rates of reaction under various conditions, and by the determination, with the aid of an isotopic tracer, of the reaction rate at the equilibrium point. Precision was achieved by extended use of ultraviolet spectrophotometry as a means of measuring initial reaction velocity and by properly standardized mass spectrometry. Results showed that: the spontaneous decomposition of oxalacetate is a first order reaction catalyzed by aspartic or glutamic acids. This confirms previously reported work carried out under different conditions (BESSMAN AND LAYNE, *Arch. Biochem.* 26: 25, 1950). The initial rate of reaction of α-ketoglutarate with aspartate increases continuously with increasing temperature between 13°–57°; energy of activation is 12,500 cal/mol; below 27° there is a short period of delay at the start of each reaction; phosphate salts exhibit a striking catalytic effect, possibly indicating their direct mediation in the reaction. Mathematical analysis of velocity data obtained at various substrate concentrations fits the possibility of ternary compound formation involving enzyme and substrates. At equilibrium, the reaction rate is only one-fourth of that predicted from determinations of initial reaction velocities in either direction.

Structure of coenzyme A. G. DAVID NOVELLI, J. DELAFIELD GREGORY, RUTH M. FLYNN AND FRANK J. SCHMETZ, JR. (introduced by FRITZ LIPMANN). *Biochemical Research Lab., Massachusetts General Hosp., Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Three phosphate groups per mole of pantothenic acid are found in our best preparations (75% pure); one appears to represent a mono-ester, while the two others are present in di-ester form, probably as a pyrophosphate bridge. With the help of the arsenolysis assay method for coenzyme A (Co A), it was discovered that prostate phosphomonoesterase inactivates Co A towards arsenolysis, while liver assay remained unaffected by such treatment. At the same time, prostate phospha-

tase liberates about one-third of the total phosphate in Co A. The remaining two-thirds require intestinal phosphatase for liberation. The adenylic acid present in Co A appears to be adenosine-5-phosphate, which was liberated from Co A in rather good yield by the use of Kornberg's potato pyrophosphatase and identified through Schmidt's deaminase test. Furthermore, a pantothenic acid-containing fragment, free of adenylic acid, was isolated by paper chromatography, and, on addition of ATP, was resynthesized to Co A in liver extracts. The sulfur component(s) linked in Co A to the carboxyl of pantothenic acid, has so far not been obtained in homogeneous form. At least part of it appears to be identical with the sulfur component in the *Lactobacillus bulgaricus* factor. The latter was identified by Snell and his group as a fragment of Co A.

Dephosphorylation of adenosine triphosphate by homogenates and intracellular constituents of rat liver. ALEX B. NOVIKOFF, LISELOTTE HECHT AND ESTELLE PODBER (introduced by HARRY G. ALBAUM). *Depts. of Pathology and Biochemistry, Univ. of Vermont College of Medicine, Burlington, Vt.*

To elucidate the nature of the enzymes in rat liver which dephosphosphorylate ATP, intracellular components were separated by differential centrifugation of 0.88 M sucrose of H_2O homogenates. ATP-dephosphorylation was then measured in the absence of added ions, in the presence of calcium ions and in the presence of magnesium ions. It was found that ATP-dephosphorylation by the nuclear fraction (free nuclei together with unbroken cells and free mitochondria) was stimulated equally by magnesium and calcium. On the other hand, ATP-dephosphorylation by the mitochondrial fraction was markedly stimulated by magnesium but not by calcium. Since the mitochondria possess about 65% of the total homogenate activity, magnesium is far more effective than calcium in stimulating the dephosphorylation of ATP by the homogenate. The products of dephosphorylation by homogenate, nuclear fraction and mitochondria fraction were followed at intervals by the method of Hitchings which permits the simultaneous determination of ATP, adenosine dephosphate and adenosine monophosphate in a sample. The results obtained will be discussed in terms of calcium and magnesium effects on the enzyme systems which may be involved. Experiments will also be described which demonstrate that, unlike the case of brain homogenates (UTTER, *J. Biol. Chem.* 185: 499, 1950), sodium ions have no stimulatory effect on ATP-dephosphorylation by rat liver, both in the presence and absence of added magnesium or calcium ions.

Crystallization of L(+) glutamic acid dehydrogenase from beef liver. JAMES ALLEN OLSON (introduced by CHRISTIAN B. ANFINSEN). *Dept. of Biochemistry, Harvard Med. School and Natl. Heart Insts., Natl. Insts. of Health, Bethesda, Md.*

Four kg. of fresh liver is homogenized with 3 volumes of ice-cold .05M K-phosphate buffer pH 7.4, and strained through a sieve. Ninety-five % ethanol is added slowly with stirring to 20% by volume (−8°C.) and the insoluble material centrifuged off. The supernatant is brought to pH 5.8 with 1M acetate buffer, pH 4.6, the solution centrifuged, and the precipitate taken up in 2 liters of .05M K-phosphate buffer, pH 7.4. The active material is precipitated again at pH 5.8 with acetate buffer, centrifuged, and the precipitate dissolved in 1 liter .05M K-phosphate buffer, pH 7.4. The solution is brought to 28% saturation with saturated Na_2SO_4 solution (temperature > 33°). The precipitate is discarded and the supernatant adjusted to 40% saturation. The resulting precipitate is dissolved in 100 ml. .05M K-phosphate buffer, pH 7.4, brought to 28% saturation with Na_2SO_4 solution, and stored overnight at +5°C. The crystalline enzyme precipitates as fine, colorless needles which can be stored for several weeks in 28% saturated Na_2SO_4 at +2°C. with only a slight loss of activity. The protein is easily recrystallized from 28% saturated Na_2SO_4 solution. This preparation gives a single peak with a slight skew in electrophoretic studies over the pH range 6–9. In the ultracentrifuge, a single hyper-sharp peak is observed. Kinetic data on the enzyme will also be presented.

Formation of fatty acids and cholesterol from radioactive acetate and glucose in rat heart, liver and hepatoma. ROBERT E. OLSON, EMILY G. HIRSCH AND MARY C. JONES (introduced by HALVOR N. CHRISTENSEN). *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Slices of cardiac muscle and liver from fed and fasted rats and of hepatoma from rats fed azo dyes were incubated in a bicarbonate buffer with either C^{14}-carboxyl-labeled acetate at 10 mM/l. or with C^{14}-evenly-labeled glucose at 5 mM/l. for 3 hours at 37°C. At the end of this period the reactions were stopped and the respiratory $C^{14}O_2$ collected. Fatty acids and cholesterol were isolated from the total slice protein. All fractions were assayed for radioactivity. In liver slices from fed animals the rate of fatty acid synthesis from acetate was approximately 10 times that from glucose and the rate of cholesterol synthesis approximately 3 times that from glucose. The ratio of fatty acid activity to cholesterol activity varied from 1–5 in different experiments. The absolute rates varied roughly in proportion to the glycogen content of the liver. In liver slices from fasting animals both fatty acid and cholesterol syntheses were markedly depressed. In cardiac

muscle slices fatty acid synthesis were much less than in liver but approximately equal rates were obtained with acetate and glucose as substrate. Cholesterol synthesis in this tissue was negligible. Despite low glycogen levels hepatoma slices demonstrated fatty acid and cholesterol synthesis from acetate and glucose. The rate of synthesis of fatty acids from glucose in tumor equalled the highest rates obtained from acetate in normal liver.

Nature of estrone conjugate in human pregnancy urine. IRVING ONESON* AND SAUL L. COHEN. *Dept. of Physiological Chemistry, Univ. of Minnesota Med. School, Minneapolis.*

Evidence is presented to the effect that a major portion of the estrone in human pregnancy urine is conjugated as the glucuronide rather than as the sulfate. Three lines of experiments support this contention. Firstly, the estrone conjugate of late pregnancy urine cannot be concentrated by use of a modified Girard procedure which permits the concentration of estrone sulfate from aqueous solutions. Secondly, the partition coefficients of the urinary estrone conjugate between butanol and aqueous solutions differs markedly from that of estrone sulfate. Thus, while estrone sulfate is only negligibly removed from butanol solutions by aqueous alkalies, the urinary estrone conjugant is largely removed by a similar extraction. Thirdly, the conjugated estrone of human pregnancy urine is almost quantitatively hydrolyzed by a preparation of calf spleen β glucuronidase under conditions which effect no hydrolysis of estrone sulfate. Estrone thus liberated from human urine has been isolated in good yield.

Influence of nitrogen mustard on in vivo sensitization reactions. LEO ORRIS AND HERMAN N. EISEN (introduced by NORTON NELSON). *Inst. of Industrial Medicine, New York Univ.-Bellevue Med. Center, New York City.*

A study was carried out on the influence of nitrogen mustard (HN-2) (methyl bis (B-chloroethyl) amine hydrochloride) on the appearance of such sensitization responses as delayed skin reactions and anaphylaxis in guinea pigs. HN-2, administered subcutaneously, 0.5 mg/kg. at 4-day intervals, produced a sustained leukopenia (about 4000/cmm.). After the first two HN-2 injections, the guinea pigs were sensitized by: *1*) intracutaneous injections of 2,4 dinitrofluorobenzene (DF); *2*) heat killed tubercle bacilli in paraffin oil; *3*) 2,4 dinitrophenyl- (D-) azo beef serum proteins given intraperitoneally in one experiment, and ovalbumin (Ea) given similarly in another experiment. After the seventh HN-2 injection, challenging tests were performed as follows: *1*) DF, applied percutaneously; *2*) P.P.D. intracutaneously; and *3*) one week later, intravenous injections of D-azo-Ea in the animals sensitized with D-azo-beef serum, or Ea in the animals sensitized with Ea. The results were: *1*) in HN-2 treated animals, DF reactions were intensely hemorrhagic, and 63% (24/38) were strongly positive; in sensitized controls the DF reactions were not hemorrhagic and only 20% (6/30) were strongly positive; *2*) tuberculin reactions were strongly positive in 8% (4/53) of HN-2 treated animals, and in 50% (15/30) of controls; *3*) fatal anaphylaxis occurred in 70% (25/36) of the HN-2 treated animals and in 80% (12/15) of the controls. In sensitized animals, nitrogen mustard, in the above dose, therefore, induced a hemorrhagic reaction to DF, apparently intensifying this response, decreased (but did not abolish) the cutaneous response to tuberculin, and produced an equivocal reduction in anaphylactic fatality.

N^{15} studies on course of synthesis of niacin in Neurospora. C. W. H. PARTRIDGE* AND DAVID M. BONNER. *Osborn Botanical Lab., Yale Univ., New Haven, Conn.*

Nutritional studies on a series of biochemical mutants of Neurospora have provided a basis for a biosynthetic scheme for the production of niacin from anthranilic acid via a single pathway involving tryptophan. To test the validity of this postulated scheme four biochemically and genetically distinct Neurospora mutants were grown on N^{15}-labeled anthranilic acid or indole and the products, tryptophan and quinolinic acid, were isolated and analysed for N^{15}. Quinolinic acid is known to be equivalent to niacin for this purpose. The ring nitrogen of tryptophan and of niacin was shown to be derived from indole and from anthranilic acid by the use of appropriate strains. It was found that alterations in growth conditions produced variations in the proportion of the nitrogen of the end products which was derived from the respective added precursors. The possible reasons for this 'leakage' effect will be briefly discussed, with special reference to the peculiar behavior of strain 39401. This strain, while unable to initiate growth on anthranilic acid, was shown to convert added anthranilic acid to tryptophan in the presence of added niacin. The combined results of these experiments indicate that niacin is synthesized from anthranilic acid via indole and tryptophan. No evidence was found for the involvement of alternate pathways or cycles in niacin formation. It is therefore concluded that the above scheme represents the sole significant path of niacin synthesis in Neurospora.

Nucleic acid content in relation to cell size in mature larval salivary gland of *Drosophila melanogaster*. ELIZABETH K. PATTERSON AND MARJORIE E. DACKERMAN (introduced by GERRIT TOENNIES). *Dept. of Genetics and Cytochemistry, Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

Using micromodifications of the trichloroacetic (Schneider) and perchloric acid (Ogur and Rosen) extraction procedures, desoxypentose (DNA) and pentose (PNA) nucleic acids were determined chemically in the larval salivary glands of *Drosophila melanogaster*. An isogenic stock, giant apricot Oregon R, was used. Comparison was made of 4 sizes of glands at the same physiological state, from male and female giant, and male and female non-giant 5-hour pupae. The means of the number of cells (150) do not differ significantly in the 4 types of gland, cell size being the variable. The nucleic acid content can be correlated with changes in other chemical constituents resulting from growth of the cells to different sizes. It had been found previously (*J. Gen. Physiol.* 32: 623, 1949) that the giant glands have twice the volume of the non-giants of the same sex; that they contain twice the alanyl-glycine (AG) peptidase and 55% more nitrogen. The comparable nucleic acid determinations show a 30% greater content of PNA in the giant than in the non-giant glands and a 55% greater total phosphorus. No significant difference could be measured in the content of DNA ($285 \pm 9 \times 10^{-6}\mu g$/nucleus) in the 4 sizes of glands. In both giants and non-giants, phosphorus and PNA are approximately $\frac{1}{3}$ higher in the female glands than in the male, similar to the relationship already found for other constituents.

Paper chromatography of acetyl salicylic acid and its metabolites. W. D. PAUL*, M. M. AL-HASHIMI* AND J. I. ROUTH. *Depts. of Medicine and Biochemistry, State Univ. of Iowa College of Medicine, Iowa City.*

Studies on salicylate metabolism have been primarily concerned with the level of salicylates in the plasma and urine following administration of aspirin or sodium salicylate. Metabolic products identified in the urine include salicyluric and gentisic acids and salicyl-glucuronides. Evidence for the presence of acetyl salicylic acid in the plasma and urine is yet inconclusive. To study the process of hydrolysis of aspirin in the body, a method of differentiating aspirin from its metabolites by paper chromatography has been developed. Several solvent mixtures were studied in an attempt to obtain distinct R_f values for acetyl salicylic acid and its metabolites. Butanol saturated with water plus 2% by volume of glacial acetic acid proved satisfactory. Using this solvent with 2.5 x 40 cm. strips of Whatman no. 1 filter paper, R_f's of 1.0 for salicylic, 0.73 for acetyl salicylic, 0.70 for salicyluric and 0.55 for gentisic acids were found. Salicylic acid could readily be differentiated from acetyl salicylic acid by R_f values, by spraying with ferric chloride, or diazotized sulfanilic acid followed by sodium carbonate solution. When these chromatograms were sprayed with *p*-dimethyl amino benzaldehyde and viewed under ultraviolet light, the compounds appeared as light yellow bands against a dark purple background. When acetyl-1-C^{14} salicylic acid was chromatographed, all of the radioactivity was concentrated at an R_f of 0.72. Plasma and urine from rabbits receiving acetyl-1-C^{14} salicylic acid exhibited radioactivity only in the acetyl salicylic acid band.

Influence of ammonia and urea upon *L. acidophilus* 4646. SHOLOM PEARLMAN AND THOMAS J. HILL (introduced by J. MUNTZ). *Inst. of Pathology and School of Dentistry, Western Reserve Univ., Cleveland, Ohio.*

Growth and glycolytic activity of *L. acidophilus* 4646 have been studied in relation to the reported inhibitory effects of ammonia and of urea upon this microorganism. Growth was retarded in nutrient broth and in a special 'synthetic' medium when ammonia was added as the phosphate or citrate, if the initial pH of the cultures was greater than 7.0. The inhibitory effect was relieved by potassium in both media, and by sodium in the 'synthetic' broth. Cells remained viable after lengthy exposures. No inhibition was observed when ammonium chloride, sulfate, or acetate were substituted, above pH 7.0, nor with any ammonium salt tested below pH 7.0. The size of the inoculum was inversely related to the degree of inhibition. Growth was inhibited moderately by 3.6% urea, and completely by 6% urea, at initial pH 7.5. In 'synthetic' broth containing either ammonium or potassium phosphate, the includion of urea was without significant inhibitory effect until its final concentration approached 3%. Glycolytic activity of washed whole-cell preparations was studied in the Warburg respirometer, at pH 7.4. Ammonium and potassium phosphates were equally effective in depressing activity, but only at concentrations greater than 0.2 M. Glycolysis was inhibited completely in 4 M urea, and only partially in lesser concentrations. Pre-incubation for 30 minutes with 4 M urea reduced glycolytic activity by 60%. No measurable amounts of ammonia were produced from urea during incubation with the cells for 3 hours. Because of the relatively high concentrations required for inhibition, the findings do not support the hypothetical role of these compounds in controlling oral *Lactobacilli*.

Isolation of allopregnanol-3(β)-one-20 and pregnanediol-3(α),20(α) from human placental extracts. W. H. PEARLMAN AND EMILY CERCEO.* *Dept. of Biochemistry, Jefferson Med. College, Philadelphia, Pa.*

Small quantities of these steroids were isolated from an acetone extract of 836 kg. of human placental tissue. Identification of allopregnanol-3(β)-one-20 was based in part on elementary analysis of the free compound, the acetate, and the semicarbazone acetate; pregnanediol-3(α),20(α) was identified by mixed m.p. determination and C,H analysis. A search was made for progesterone. A ketonic fraction (111.5 mg.) was obtained, which from its absorption maximum at 240 mμ, was estimated to contain 53 mg. of 'progesterone;' this measurement is not specific however for progesterone but is indicative of α, β unsaturated ketones. Chromatography of this fraction yielded a minute quantity of crystals (from methanol), m.p. 209°–211°, $[\alpha]_D$ + 248° ± 22°(ethanol), λ_{max}^{alc} 236 mμ, $E_{1cm.}^{1\%}$ = 483. A non-crystalline eluate was also obtained, $[\alpha]_D$ + 148° ± 4°(ethanol), λ_{max}^{alc} 240 mμ, $E_{1cm.}^{1\%}$ = 441. Compare with progesterone, m.p. 128°, $[\alpha]_D$ + 192°, λ_{max}^{alc} 240 mμ, $E_{1cm.}^{1\%}$ = 509. The presence of progestin in placentas has been demonstrated by bioassay by some investigators, although others have often obtained negative results. There is, however, other evidence that the placenta is intimately linked with progesterone metabolism and with the excretion of pregnanediol in the urine. This study is being continued. *Acknowledgments:* We are indebted to Dr. R. H Barnes (Sharp and Dohme, Inc.) and to Dr. W. J. Haines (Upjohn Co.) for the placental extracts. This investigation was supported by research grants from the National Research Council, Committee on Research in Endocrinology and from the National Institutes of Health, U. S. Public Health Service.

Coupling of hydrogen transport with phosphorylation in *E. coli* extracts. G. B. PINCHOT* AND E. RACKER. *Dept. of Microbiology, New York Univ. College of Medicine, New York City.*

Extracts of *E. coli* obtained by sonic disintegration were studied for $DPNH_2$ oxidase activity by measuring the disappearance of $DPNH_2$ spectrophotometrically at 340 mμ. The specific activity of extracts from cells grown in neopeptone broth was three-to fivefold that of cells grown in a defined media with glucose as carbon source. The oxidase activity of bacterial extracts was not inhibited by concentrations of KCN and BAL which markedly inhibited heart muscle preparations of $DPNH_2$ oxidase. Coupling of phosphorylation with oxidation was studied by trapping ATP (formed from ADP) with hexokinase and glucose, and measuring oxygen and phosphate uptake. Without added substrate there was some uptake of oxygen and phosphate, which was increased by the addition of alcohol or acetaldehyde and markedly accelerated by the addition of alcohol and yeast alcohol dehydrogenase. With alcohol as substrate the P/O ration was 0.8 to 1.0, with acetaldehyde about 1.5. Addition of acetate did not increase the oxygen uptake. Both oxygen and phosphate uptake were DPN dependent. Extracts kept frozen for 1 week were still capable of coupled phosphorylation. High-speed centrifugation (3 hours at 18,000 R.P.M.) removed some of the activity but most remained in the supernatant. By treatment of the extracts with Dowex-1 Cl and fractionation with ammonium sulfate, a preparation was obtained which did not oxidize acetaldehyde but was capable of coupling alcohol oxidation with phosphorylation. A P/O ration of 0.7 was obtained under these conditions.

Enzymatic incorporation of C^{14} carbonate into acetoacetate in the presence of various substrates. G. W. E. PLAUT (introduced by HENRY A. LARDY). *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

The incorporation of C^{14} carbonate into the carboxyl group of acetoacetate by rat liver *homogenate* systems containing malonate varied considerably depending on the source of acetoacetate. The results obtained with the different substrates fell into four categories: *1*) Pyruvate, crotonate, butyrate, caproate, heptylate, caprylate, and pelargonate led to a definite net formation of acetoacetate and an accompanying increased incorporation of $C^{14}O_2$ into the carboxyl group of acetoacetate. *2*) Net formation of acetoacetate was observed with DL-β-hydroxybutyrate and valerate. However, very little or no radioactivity could be detected in acetoacetate produced from these substances. The lack of incorporation of C^{14} into acetoacetate generated from β-hydroxybutyrate here is reminiscent of the failure of this compound to act as an intermediate in the oxidation of butyrate to acetoacetate. *3*) Isovalerate led to a pronounced increase of the radioactivity of carbon 1 of acetoacetate but did not cause a demonstrable net formation of this ketone body by *homogenates*. This phenomenon suggests that isovalerate yields a molecular fragment to which a large amount of $C^{14}O_2$ can be added to yield acetoacetate of very high specific activity. Coon has recently reported the incorporation of $C^{14}O_2$ into the carbon 1 position of acetoacetate derived from isovalerate. Experiments here with rat liver *slices* demonstrate a *stoichiometric* relationship between the conversion of the isopropyl group of isovalerate to acetoacetate and the incorporation of C^{14} carbonate into carbon 1 of acetoacetate. *4*) Neither an appreciable net production of acetoacetate nor a stimulation of C^{14} incorporation could be found with added 3-phosphobutanoate, leucine, valine, isobutyrate, and isopropanol.

Attempts to identify a fraction isolated from an enzymatic digest of desoxyribonucleic acid. JOSEPH L. POTTER*, K. D. BROWN* AND M. LASKOWSKI. *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.*

Desoxyribonucleic acid from calf thymus was prepared according to Hammarsten (*Biochem. Ztschr.* 144: 383, 1924). It was digested with crystalline desoxyribonuclease prepared according to Kunitz (*Science* 108: 19, 1948). The end products of the reaction were chromatographed under the conditions described by Cohn (*J. Am. Chem. Soc.* 72: 1471, 1950). Dowex #1, Amberlite IR-400, and Amberlite IR-4B were used. In all cases only a small part of the total digest was eluted from the resin by means of dilute HCl at pH 2.5. The fractions eluted under these conditions accounted for 2–4% of the total phosphorus, and had a maximum absorption at about 270 mμ. No free bases were detected in any of these fractions. Using the paper chromatographic technique of Carter (*J. Am. Chem. Soc.* 72: 1466, 1950) for resolution of nucleotides, at least three components were indicated. The Rf values found were 0.60, 0.73, 0.82 in alkaline phosphate, and 0.67, 0.76, 0.84 in acid phosphate. On the basis of phosphorus content, the presence of nucleosides was excluded.

Titration of a succinoxidase component by antimycin. V. R. POTTER AND A. E. REIF.* *McArdle Memorial Lab., Med. School, Univ. of Wisconsin, Madison.*

Further work has been done on the recently discovered antibiotic Antimycin, which virtually titrates an essential component of the succinoxidase system in rat tissue (cf. AHMAD, SCHNEIDER AND STRONG, *Arch. Biochem.* 28: 281, 1950). The inhibitor is apparently quite specific and may have considerable value for enzyme studies. For a wide range of organs, an Antimycin concentration of about 8×10^{-8} g. causes 50% inhibition with an amount of tissue sufficient to give a succinoxidase activity of 100 μl. oxygen per 10 minutes, as measured by the usual homogenate technique in the Warburg apparatus at 38°C. Since the succinoxidase content of tissues varies widely, this means that Antimycin titrates an enzyme component rather than non-specific tissue constituents. Antimycin appears to be slowly metabolized by liver homogenates, since a restoration of succinoxidase activity can be observed at critical levels of the inhibitor, if sufficient time is allowed. Antimycin has no effect on cytochrome oxidase or succinic dehydrogenase activity, indicating that Antimycin inhibits the Slater factor as currently defined. Antimycin (3×10^{-7} g.) completely inhibits the oxidation of all Krebs cycle substrates by rat liver homogenates (90 mg. liver in 3 ml.) in a system fortified with adenosine triphosphate. However, with succinate, the initial inhibition is progressively reversed as Antimycin is metabolized. Antimycin (4×10^{-7} g.) has no effect on the anaerobic glycolysis of tumor tissue (30 mg. in 3 ml.).

Serum bilirubin. BETTY KING PROFFITT* AND DEMPSIE B. MORRISON. *Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis.*

In extending our studies on the factors affecting the diazotization of bilirubin (*Federation Proc.* 8: 239, 1949) we have calculated the ratios of direct reacting to total bilirubin on the sera of 66 adult patients with jaundice. Included in this series were patients with cirrhosis, massive hepatic necrosis, hepatitis (recurrent, postoperative, and homologous serum), metastatic carcinoma obstructive jaundice (cholecystitis, cholelithiasis, carcinoma, and atresia), and myelogenous leukemia. Serum bilirubin ranged from 1.5–27.0 mg. %. The average direct/total ratio was 0.73 (S.D. 0.075). Twelve samples of 'jaundiced sera' covering approximately the above range of bilirubin were prepared by adding to pooled normal sera human bladder bile. Seven different samples of bladder bile were used. The average direct/total ratio for this series was 0.70 (S.D. 0.066). The ratios in the clinical and artificial series are not significantly different. There was no significant or consistent correlation between the clinical diagnosis or the degree of hyperbilirubinemia and the ratio of direct to total bilirubin. When bilirubin is diazotized in aqueous solution, at the pH and concentration of nitrate normally used, less color is formed than when diazotization is carried out in 50% methanol. This fact and the relative constancy of the direct/total ratios indicate that only one form of bilirubin was present in the sera of these patients.

Comparison of diphosphopyridine nucleotide (DPN) with desamino DPN in various enzyme systems. MAYNARD E. PULLMAN (introduced by SIDNEY P. COLOWICK). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

The recent finding of Kaplan *et al.*, reported elsewhere in this volume, of an enzyme which deaminates DPN, made it of interest to compare the activity of desamino DPN with DPN in various DPN linked systems. Schlenk *et. al.* (Ber. 71: 1471, 1938) using chemically prepared desamino DPN found that this compound was one-tenth as active as DPN towards yeast alcohol dehydrogenase. This observation was confirmed; however, no difference in activity of these two compounds was observed toward purified horse liver alcohol dehydrogenase. When compared in the lactic dehydrogenase system, there again was no apparent difference in the rate of reduction of these

dinucleotides. In the acetaldehyde dehydrogenase system, prepared from beef liver as described by Racker (*J. Biol. Chem.* 177: 883, 1949), it was found that desamino DPN was one-seventh to one-tenth as active as DPN, depending on the concentration of nucleotide. In all but the last mentioned system there was no apparent difference in the dissociation constant (K_s) of these compounds. However, in the aldehyde dehydrogenase system K_s was approximately ten times as high for desamino DPN. Desamino DPN was more active than DPN in the diaphorase system of rat liver mitochondria. No difference in activity was observed in the rate of oxidation of reduced triphosphopyridine nucleotide by these compounds in an extract of *Pseudomonas* (Colowick *et. al.* this volume). Measuring the activity of potato pyrophosphatase towards DPN and desamino DPN revealed no apparent difference in the rate of the splitting of these nucleotides. However, the *Neurospora* DPNase was one-fiftieth as active toward desamino DPN.

Ratio of free to total prothrombin in various types of hypoprothrombinemia. ARMAND J. QUICK AND CLARA V. HUSSEY.* *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.*

Experimental evidence has been obtained showing that in man, prothrombin in plasma exists partly free and partly inactive (prothrombinogen). The one-stage prothrombin test determines specifically the active fraction. To estimate total prothrombin, platelet-poor native plasma (obtained by the use of silicone-coated apparatus and high centrifugation) is allowed to clot and the prothrombin of the serum determined. In the absence of platelets, little active thromboplastin is formed, therefore only a trace of prothrombin is consumed; but all of the prothrombinogen, under these conditions, is converted to active prothrombin. By determining the prothrombin of the serum, a quantitative estimate of the total prothrombin is obtained. In one type of congenital hypoprothrombinemia, only free prothrombin is reduced while the total is normal. This condition is hereditary and occurs in both sexes. In the second congenital type, which is also familial, both prothrombin and prothrombinogen are below normal. On giving dicumarol, the free prothrombin decreases first whereas no drop of prothrombinogen may occur for several days. After a prolonged dicumarol therapy, an equilibrium between the free and total appears to become established. Occasionally a decrease of total prothrombin but a normal concentration of free prothrombin is observed in normal subjects.

Hydrolysis of hyaluronic acid by pneumococcal hyaluronidase. MAURICE M. RAPPORT AND ALFRED LINKER.* *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic, Presbyterian Hosp., New York City.*

When hydrolysis of hyaluronic acid by pneumococcal hyaluronidase is complete, 50–75% more reducing sugar is formed than on completion of the hydrolysis by testicular enzyme. The action of pneumococcal enzyme on the product of testicular enzyme action leads to a comparable increase in reducing power of the reaction mixture. These facts have heretofore been interpreted as indicating that pneumococcal enzyme split the substrate to monosaccharides, while the end-product of testicular enzyme action was a disaccharide. By the application of a recently developed analytical method (RAPPORT, MEYER AND LINKER. *J. Am. Chem. Soc.* in press) it has now been found that monosaccharides are not formed on hydrolysis by pneumococcal enzyme. Chemical analysis of the isolated, amorphous final product shows it to be composed of equimolar quantities of acetylglucosamine and glucuronic acid. The free reducing group is on the amino sugar residue. The specific rotation is negative. These properties are the same as those observed for the product of testicular enzyme action. However, the reducing power of the pneumococcal enzyme product is much higher than that of the testicular enzyme product, its migration on a paper chromatogram is much faster and it is completely eluted from the carbon column by 15% ethanol. These properties are in close accord with those attributable to a disaccharide. Pneumococcal hyaluronidase hydrolyzes hyaluronic acid more rapidly than it hydrolyzes the product formed by testicular enzyme. This observation indicates that the mechanisms of action of pneumococcal and testicular hyaluronidase are different. (This work is supported by the Helen Hay Whitney Fndn.)

Nitrogen metabolism of hogs infected with hog cholera virus. ELWOOD REBER,* C. K. WHITEHAIR* AND ROBERT MACVICAR. *Oklahoma Agricultural Exper. Station, Stillwater.*

The effect of virus disease on nitrogen metabolism of swine has been investigated using hog cholera virus. Animals were fed a semi-purified ration supplemented with minerals and vitamins from 2 days of age. Preceding the innoculation with hog cholera virus, casein, acidhydrolyzed casein supplemented with methionine and cystine, or zein-gelatin combinations were used as protein sources. Animals were infected by injection of a virulent source of virus and nitrogen equilibrium determined during the ensuing period. Animals receiving an adequate dietary protein died within

4–6 days with typical symptoms and lesions of acute hog cholera. Nitrogen balance throughout the course of the infection was positive in four out of five cases. Animals fed acid-hydrolyzed casein or zein-gelatin rations for some time prior to innoculation were not as acutely affected, and 6 out of 8 animals recovered. Animals succumbing showed a more chronic symptomatology, lived over twice as long, and had milder gross tissue changes. No differences between infected animals and corresponding controls with respect to the following was observed: hemoglobin; plasma protein; plasma tryptophan; riboflavin, niacin and pantothenic acid content of liver. Tissue glutamine levels were not elevated in the infected animals, tending to confirm the observed positive nitrogen balance. A moderate leukopenia was noted in the infected animals.

Effect of transplanted tumors on the nucleic acid content of mouse tissues. D. V. N. REDDY* AND LEOPOLD R. CERECEDO. *Dept. of Biochemistry, Fordham Univ., New York City.*

A quantitative study of nucleic acids was carried out during the development of transplanted Crocker sarcoma 180, and melanoma (S-91), in mice of the dba strain. Tumor, liver, lung, kidney, and spleen were analyzed at one-week intervals for pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA). In the case of the animals with the transplanted sarcoma the nucleic acid content of liver, kidney and lung sharply increased at the end of 3 weeks, while the slow-growing melanoma had a similar effect on the host after 6 weeks. The maximum increase of nucleic acids over the normal in various tissues was as follows: PNA-liver, 50%; lung, 64%; kidney, none; DNA-liver, 36%; lung, 86%; kidney, 65%. In either case spleen values remained unaltered. The ratio PNA/DNA, which is approximately unity in the initial stage falls to a low value of 0.53 in a 9-week-old-melanoma. Caspersson and Santesson (*Acta Radiol. Suppl.* 46: 1, 1942) interpreted similar results as due to a drop in PNA of the necrotic portion. However, we have found that both PNA and DNA show a gradual drop not only in the necrotic, but also in the non-necrotic part of the tumor. The 3rd week values of 57 mg/gm. dry tissue for PNA, and 55 mg/gm. dry tissue for DNA dropped to 23 and 44, respectively, at the end of 9 weeks. The necrotic part of the same melanoma showed the corresponding values of 17 and 31 per unit weight of dry tissue. (Aided by a grant from the Damon Runyon Memorial Fund).

Electrophoretic studies of human serum albumin at pH 4.5. JOHN G. REINHOLD AND LOGAN GILMAN.* *Pepper Lab. of Clinical Medicine, Hosp. of Univ. of Pennsylvania, Philadelphia.*

The two components detected in human serum albumin subjected to electrophoresis in sodium acetate-sodium chloride buffer of pH 4.5., have been studied. One, almost stationary at the starting boundary comprises 50–70% of the total albumin, the other constituting the remaining area, migrates toward the anode. Their counterparts may be identified in serum under the same conditions. Further analysis indicates that the stationary component consists of albumin electrophoretically homogeneous at pH 8.6. The possibility that the second component is an artefact has been excluded. The relative amounts of the two albumin components have been studied in a number of pathological conditions. Some evidence of characteristic variations has been obtained.

Hydrogenation of polyunsaturated fatty acids by the ruminant. RAYMOND REISER (introduced by KENNETH A. KUIKEN). *Texas Agricultural and Mechanical College System College Station.*

The body fats of sheep and cattle contain very small quantities of linolenic acid even on a grass ration containing considerable quantities of this acid, while non-ruminants (horses) on the same regime deposit appreciable amounts. In order to test the hypothesis that this difference may be due to hydrogenation of the linolenic acid by bacterial action in the rumen, rumen contents were incubated with emulsions of linseed oil. After 4 days of incubation the linolenic acid content of the fat of the rumen contents—linseed oil mixture was reduced from approximately 30% to approximately 5%. Linoleic acid increased proportionately, but the other acids remained unchanged. Autoclaved controls underwent no hydrogenation.

Bioassay procedure for the unidentified dietary factor related to xanthine oxidase. DAN A. RICHERT* AND W. W. WESTERFELD. *Biochemistry Dept., Syracuse Med. College, Syracuse, N. Y.*

Feeding weanling rats a purified diet containing 0, 2, 5, 10 or 15% liver residue gave progressively increasing levels of intestinal xanthine oxidase activity that ranged from 6–33 Cmm O_2/20 min/283 mg. This intestinal response provided a satisfactory biosssay curve for the unidentified dietary factor related to xanthine oxidase. The liver residue could be added to a 21% casein diet at the expense of glucose or it could be incorporated in diets containing a constant total protein content of 31%. The diets could be fed directly to weanling rats for 2 weeks, or the intestinal enzyme could first be depleted by feeding a purified diet for 1–2 weeks, and the test diets then fed for 2 or 7 days. Relatively small differences in the bioassay curve

were produced by such variations. The following were good sources of the unknown factor: kidney, liver residue, spleen, aureomycin mash, soy bean flour, cerophyl, and buttermilk. Duodenum and milk were moderate sources, while heart, pancreas and yeast were poor. Crystalline aureomycin was weakly positive or negative. All of the pure vitamins including folic acid, xanthopterin and B_{12} were negative. Cystine and methionine were negative. Purified xanthine oxidase from cream or skim milk was positive. Based upon distribution the possible identity of this factor with Ershoff's antithyrotoxic factor is indicated.

Concentrative uptake of glycine and alanine by erythrocytes. THOMAS R. RIGGS,* HALVOR N. CHRISTENSEN AND NANCY E. RAY.* *Dept. of Biochemistry and Nutrition, Tufts College Med. School, Boston, Mass.*

Nucleated duck erythrocytes contained three to five times as high a concentration of apparently free glycine as the plasma. Most, if not all, of this glycine diffused out when the cells were washed for several hours with a number of changes of saline. No glycine-binding could be detected by equilibrium dialysis of a hemolysate prepared by freezing and thawing. The same applied to the alanine and glycine of human erythrocytes. When duck erythrocytes were suspended in saline concentration gradient, the increase of the cellular level being greater than the extracellular; with varying glycine levels the concentration gradient remained approximately constant at 2 mg. glycine N per 100 gm. water. At extracellular concentrations below those of plasma, the distribution ratio rather than the gradient tended toward constancy. The uptake was maximal at about 38°, much lower gradients being obtained at 25° or 50°. When blood was refrigerated, glycine passed out of the cells into the plasma, but upon incubation the glycine was taken up again by the cells. The uptake was partially inhibited by oxygen lack, but was surprisingly resistant to certain respiratory enzyme inhibitors, notably cyanide. The assimilative process was inhibited when half of the sodium of the medium was replaced by potassium ion. Concentrative uptake was also observed for glycine and L-alanine with the human erythrocyte. D-alanine was taken up very little if at all.

Glutamic acid decarboxylase in brain. EUGENE ROBERTS AND SAM FRANKEL.* *Wernse Lab. of Cancer Research, Washington Univ. School of Medicine, St. Louis, Mo.*

γ-Aminobutyric acid, which is present in the free form in large amounts in brain, is formed by α-decarboxylation of glutamic acid by an enzyme which is present in brain in uniquely high concentrations. The enzymatic activity was demonstrated in homogenates and acetone powders. The enzyme behaves like a typical decarboxylase, requiring pyridoxal phosphate as coenzyme and being inhibited by semicarbazide and hydroxylamine. Conditions were established for manometric assay in which the reaction was of zero order and the activity was proportional to the quantity of enzyme employed. The PH optimum of the enzyme from mouse brain was found to be between 6.4 and 6.5. The Michaelis-Menten constant was estimated to be 6.4×10^{-3} M. Of a large number of amino acids and amino acid derivatives tested, only L-glutamic acid was decarboxylated by the enzyme. Pyridoxal phosphate was highly specific as a coenzyme, pyridoxal, pyridoxine, 4-desoxypyridoxine, 4-methoxymethylpyridoxine, and pyridoxamine being inactive in the system. Experiments with the decarboxylase as a test system showed that synthesis of codecarboxylase takes place when pyridoxal and ATP are added to a homogenate of fresh brain in the presence of fluoride. By measuring the activity of homogenates of rat brain in the presence and absence of added coenzyme, it was shown that the glutamic acid decarboxylase activity of pyridoxine-deficient animals was only one-half that found in the controls, although the quantity of apoenzyme was not reduced. Refeeding of pyridoxine produced a rapid return of the decarboxylase activity to normal.

Influence of adrenal cortex on serum and liver protein regeneration in the partially-hepatectomized rat. SIDNEY ROBERTS. *Dept. of Physiological Chemistry, School of Medicine, Univ. of California, Los Angeles.*

Alterations in the serum protein pattern occurring during the first few days after partial removal of the liver in the rat have been reported earlier (ROBERTS AND WHITE. *J. Biol. Chem.* 180: 505, 1949). In an extension of these studies, the influence of the level of adrenal cortical activity on this phenomenon was investigated. The untreated animal exhibited a decline in total serum protein concentration for about 24 hours following partial hepatectomy. After this time, there was a gradual return to a normal level. In rats adrenalectomized 4 days before hepatectomy, the serum protein levels fell much more rapidly and in a more sustained fashion. Chronic treatment of similar animals with ACTH prior to and following partial hepatectomy almost completely prevented these changes. Electrophoretic analyses revealed a decline mainly in albumin and α_1-globulin in the control group, a more generalized fall in albumin and all the globulins in the adrenalectomized group, and a redistribution of albumin and globulin levels in the ACTH-treated animals. The differences in serum protein regeneration were well correlated with the rate of early liver regen-

eration in the three groups of rats. This rate was greatest in the ACTH-treated animals and least in the adrenalectomized animals.

Influence of adrenal cortex on the aerobic metabolism of normal and malignant lymphoid tissue. SIDNEY ROBERTS AND ABRAHAM WHITE. *Dept. of Physiological Chemistry, School of Medicine, Univ. of California, Los Angeles.*

Investigations have been carried out on the effects of the adrenal cortical secretions on the aerobic metabolism *in vitro* of normal lymphoid tissue and lymphosarcomata of CBA mice and Sprague-Dawley rats. The following biochemical processes have been followed: oxygen consumption, glucose utilization, lactic acid production, release of protein, amino acids, and inorganic phosphate. The release of protein to a serum medium by normal and malignant rat lymphoid tissue was directly correlated with the level of adrenal cortical activity in the animal supplying the tissues. Electrophoretic analyses revealed that the protein released in all cases traveled with the electric mobility of β-globulin; albumin disappeared from the serum medium coincidentally. The disappearance of albumin was greater in incubates of tumor tissue than of normal lymphoid tissue. None of the other metabolic processes measured were affected by the adrenal cortical secretions in normal lymphoid tissues of rat and mouse. However, surviving lymphosarcoma tissue from rats pretreated with ACTH exhibited an increased rate of glucose utilization and a decline in release of amino acids. These differences were not associated with a change in total oxidative metabolism in the tumor tissue.

Microdetermination of phospholipids and sphingolipids in brain. ELI ROBINS* AND OLIVER H. LOWRY. *Depts. of Pharmacology and Neuropsychiatry, Washington Univ., St. Louis, Mo.*

In connection with a study of the quantitative histochemistry of brain, relatively simple and rapid micromethods are being elaborated for the measurement of cephalins, lecithin, sphingomyelin, and total sphingolipids in 25 μg. of brain (less than one μg. of a particular lipid). The lipids are extracted from TCA precipitates, in tubes of 2 mm. bore, with buffered alcohol and isopropyl ether (total volume of solvents approximates 0.05 ml.). The Schmidt method for quantitative separation of sphingomyelin from other phospholipids has been adapted for smaller scale work by extraction of the cold-alkali hydrolysate (0.01 ml.) with octanol (0.01 ml.) instead of by precipitation with TCA. The sphingomyelin is measured directly by the P of the octanol extract. The total cephalins are determined colorimetrically by treating the aqueous phase with fluorodinitrobenzene (FDB). This forms the dinitrophenyl derivatives of those lipids which contain primary amino groups. (Alternatively, cephalins may be measured on the unhydrolyzed lipid extract without sphingomyelin removal.) Lecithin is calculated from the P of the aqueous phase after subtracting the P attributed to the cephalins. (A modification of the Fiske-Subbarow phosphorus method permits measurement of 0.005 μg. P.) The method for total sphingolipids (still in process) involves 6 N HCl hydrolysis of the octanol extract, and reaction of the freed amino groups with FDB. The simplicity of the proposed microprocedures possibly recommends them for use in lipid studies on a macro scale. (Supported in part by a USPHS Fellowship (NIMH), and an American Cancer Society Grant.)

Deamination of amino acids by *Brucella*. W. G. ROESSLER, F. A. LEONARD AND L. H. GRAF (introduced by C. R. BREWER). *Camp Detrick, Frederick, Md.*

Conway microdiffusion equipment and the Warburg apparatus have been employed in studies on the deamination of amino acids by resting cells of the *Brucella suis, melitensis,* and *abortus* species. The avirulent strain of *B. abortus* (A19) was less active in deaminating amino acids than the other species tested. *B. suis* attacked the guanido group in arginine and the ϵ-amino group in L-lysine. The rate of deamination of L-arginine *B. suis* was linear until approximately 75% of the nitrogen was converted to ammonia, indicating that 3 out of the 4 nitrogens in arginine were deaminated at a constant rate. Studies on the deamination of DL-ornithine indicated that it was attacked. Urea was rapidly and completely deaminated. These data indicate that the residue from the deamination of arginine probably contains the δ-amino group. Glycine, valine, proline, histidine, serine, tryptophane, glutamic acid, leucine, asparagin, phenylalanine, isoleucine, and tyrosine were also deaminated by *B. suis*. Attempts to identify the products of deamination of amino acids by chromatography and by the preparation of derivatives are reported.

Utilization of purine nucleotides for nucleic acid synthesis by the rat. PAUL M. ROLL AND IRVING WELIKY.* *Sloan-Kettering Inst. for Cancer Research, New York City.*

Ribonucleotides were isolated from an alkaline hydrolysate of yeast nucleic acid which had been labeled biosynthetically with N^{15}. The separation was accomplished by precipitating the purine nucleotides with Cu^{++} and HSO^{-3}. The adenylic and guanylic acids were separated by means of the differential solubility of their barium salts and the pyrimidine nucleotides were fractionated

by precipitating cytidylic acid with phosphotungstic acid. The labeled purine nucleotides were administered, parenterally, to adult Sherman strain rats at a level of 0.4 mM/kg/day for 3 days. Sodium nucleates were isolated from the viscera of the animals. These were treated with 1 N NaOH to hydrolyze the PNA. The DNA was recovered by acid precipitation according to a modification of the Schmidt and Thannhauser procedure and PNA nucleotides were precipitated as barium salts. Each fraction was hydrolyzed with perchloric acid and the purines and pyrimidines were separated by the use of a column of Dowex-1 ion-exchange resin. It was found that 3% of the adenine and 1% of the guanine, but practically none of the pyrimidines of the PNA, had been formed from the injected adenylic acid. Following the administration of guanylic acid it was found that 2% of the guanine but none of the adenine or pyrimidines of the PNA had been derived from the injected nucleotide. There was not more than a trace of incorporation of adenylic or guanylic acids into the DNA.

Specificity of hemolytic reaction in tocopherol-deficient erythrocytes. CATHARINE S. ROSE* AND PAUL GYÖRGY. *Nutritional Service of Dept. of Pediatrics and Gastro-intestinal Section of Med. Clinic, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Red blood cells of vitamin E-deficient rats are hemolyzed by dialuric acid. Alpha tocopherol, whether it has been fed to the rat or incubated with the blood cells *in vitro* protects them against hemolysis. (*Blood* 5: 1072, 1950). Various substances have been studied with respect to their protective or hemolytic effect in this reaction. *In vitro*, protection was afforded by beta, gamma and delta tocopherols, highly substituted hydroquinones and estrogenic substances. *In vivo*, only the tocopherols gave demonstrable protection. In both cases alpha was the most active of the tocopherols, followed successively by beta, gamma and delta, the ratio of the activities being, *in vivo* 1 : 0.1 : 0.04 : 0.03 and *in vitro* 1: 0.3 : 0.2 : 0.15. During the autoxidation of dialuric acid hydrogen peroxide is formed. To determine whether this might be the actual hemolyzing agent catalase was added to the *in vitro* system. Hemolysis was prevented by active but not by heated catalase. Hemolysis of normal blood by hydrogen peroxide has been reported (BLUM, *Physiol. Rev.* 12: 23, 1932). The present studies show that the hemolyzing activity is directly related to the tocopherol content of the erythrocytes. Under the same experimental conditions tocopherol-deficient cells were almost completely hemolyzed while those with an adequate content of tocopherol were protected. Under these same conditions erythrocytes of normal human adults were protected while those of new-born infants showed hemolysis up to 50%.

Changes in concentration and type of hepatic alkaline phosphatase due to dietary deficiency and regeneration. OTTO ROSENTHAL, JAMES C. FAHL* AND EDWIN J. WELLS.* *Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

It was previously reported by us (*Federation Proc.*) 9: 220, 1950) that the alkaline phosphatase content of rat liver increases on protein depletion. We have now observed that we have been dealing with two alkaline phosphatases probably corresponding to the phosphatases I and II of Cloetens (*Enzymologia* 6: 46, 1939) and characterized by differential sensitivity to magnesium activation and cyanide inhibition. The present experiments were done upon liver tissue and plasma from normal protein-fed, or 7 days fasted rats, animals one day after partial hepatectomy, and 2 weeks protein-depleted groups. The percentage of phosphatase II, i.e. of the cyanide-sensitive portion of the total alkaline phosphatase activity at optimal mg.-concentration was as follows: plasma (all groups) 94; liver (normal or fasting) 29; liver (protein depletion or regeneration) 62. In protein depletion as well as after partial hepatectomy there was a striking rise in phosphatase II accounting entirely for the increased phosphatase activity of depleted liver, and largely, though not exclusively, for the increase during regeneration. Since phosphatase II is apparently the major component of plasma phosphatase it seems possible that the latter accumulates in the liver whenever its supply with the blood is less reduced than the effective liver mass. This does not occur in fasting where both components of liver phosphatase decrease proportionally to the hepatic protein. (This work was done under contract with the Dept. of the Army.)

Phosphorus compounds in peripheral nerve during wallerian degeneration. R. J. ROSSITER, J. E. LOGAN* AND W. A. MANNELL.* *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*

The wet weight and the concentration of total phosphorus, acid-soluble phosphorus, lipid phosphorus and desoxypentosenucleic acid phosphorus (method of Schmidt and Thannhauser, *J. Biol. Chem.* 161: 83, 1945) were determined in the degenerating sciatic nerve of a series of 21 cats at intervals of time from 2 to 96 days after nerve section. The intact nerve of the opposite side served as a control. The wet weight and lipid phosphorus followed a course previously described from this laboratory (*Biochem. J.* 45: 500,

1949). The concentration of acid-soluble phosphorus remained unchanged for the first 4 days after the nerve section and then rose sharply to a maximum at 8 days, which was maintained until 16 days. By 32 days the concentration of acid-soluble phosphorus had commenced to fall and by 96 days it was less than in the control nerves. The concentration of desoxypentosenucleic acid changed little during the first 2 days, but it then rose sharply, reaching a maximum in the 16- to 32-day period. By 96 days the concentration had fallen, but it was still greatly in excess of the control values. Total nucleic acid, pentosenucleic acid, desoxypentose-nucleic acid and 'phosphoprotein' were also determined by the method of Schneider (*J. Biol. Chem.* 161: 293, 1945) and pentose-nucleic acid was determined by the method of Schmidt and Thannhauser. There are reasons to believe that in tissue from the nervous system all these figures represent an overestimate. The results are discussed in relation to Schwann cell proliferation and macrophage infiltration in degenerating nerve.

Free amino acids and other ninhydrin positive substances in rapidly growing tissues of the rabbit. GEORGE ROUSER AND GARSON H. TISHKOFF (introduced by ELMER STOTZ). *Depts. of Biochemistry and Pharmacology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*

Paper chromatographic studies have shown that tumor and embryonic tissues have a similar free amino acid pattern. For this reason, it seemed desirable to compare these tissues with normal adult rapidly growing tissue. Polymorphonuclear leucocytes (from peritoneal exudates) and lymphocytes were studied by two dimensional paper chromatography after treatment of the homogenized tissue with 78% alcohol. The chromatograms were very much like those for tumor and embryo tissue. The absence of the essential amino acids and the presence of glutamic acid, underglutamic acid, alanine, valine, leucine, aspartic acid, glutathione, taurine and an unidentified phosphorus containing nitrogen compound is the characteristic pattern. In addition beta alanine, glutamine and serine occur in smaller quantity. Intestinal mucosa, a rapidly growing tissue, was also found to follow this pattern quite closely. The liver shows some deviation, although it follows the pattern in general. The phosphorylated compounds in the chromatograms were studied by administering P^{32} to an animal before removing the appendix (lymphocytes). The chromatograms were developed with ninhydrin and an autoradiograph was made. The substance underglutamic was isolated using P^{32} and shown to be ethanolamine phosphoric acid in confirmation of the work of Awapara *et al.* (*J. Biol. Chem.* 183: 545–548). The presence of ethanolamine phosphoric acid and the as yet unidentified phosphorylated nitrogen compound indicate that phospholipid fragments may also be characteristic for rapidly growing tissue.

Intense reducing system of rabbit polymorphonuclear leucocyte. GEORGE ROUSER (introduced by ALEXANDER DOUNCE. *Dept. of Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*

A system that reduces indophenol dyes aerobically and anaerobically is more intense in the polymorphonuclear leucocyte than in other tissues. The system is so pronounced that cytochrome oxidase cannot be assayed by the method of Smith and Stolz (*J. Biol. Chem.* 179: 891–902, 1949) which can be used for other tissues. Homogenizing the cells lowers the reducing intensity to about one-tenth that of intact cells. The *in vitro* degeneration of this reducing ability in the intact cell at 37°C. has been studied using cells collected from the peritoneal cavity by the method of de Haan. The cells were suspended in an isotonic solution of sodium chloride, phosphate buffer (pH 7.4) and heparin. The reducing ability was measured quantitatively by following in the Klett colorimeter the reaction rate of a suspension containing approximately 10,000 cells/mm.^{3} The reducing ability declines to a stable value of 5–10% of the original in 25–30 hours and follows a straight line course when reducing ability is plotted against time. Morphologically there is clumping and disintegration of the cells as the reducing ability declines. At 5°C. the system declines to a minimum value in about 50 hours. Centrifugation at speeds ordinarily used in blood cell separations has little or no effect on the disappearance of the reducing system. Studies are in progress to determine the effect of x-ray irradiation upon the system.

Phosphorolysis of nicotinamide riboside. JOHN W. ROWEN* AND ARTHUR KORNBERG. *Natl. Insts. of Health, Bethesda, Md.*

An enzyme has been purified from extracts of beef liver acetone powder which catalyzes the phosphorolytic cleavage of nicotinamide riboside according to the equation: nicotinamide riboside$^+$ + phosphate → nicotinamide + ribose-1-phosphate + H^+. The reaction was followed by measuring the disappearance of nicotinamide riboside and the appearance of acid-labile phosphate and nicotinamide. The latter was determined by a sensitive (0.005 micromoles/ml.) spectrophotometric method which utilized the density change accompanying a shift from neutral to acid pH. No reaction occurred in the absence of phosphate. The enzyme did not attack methyl nicotinamide, nicotinamide ribose-5′-phosphate, diphosphopyri-

dine nucleotide and triphosphopyridine nucleotide but did act upon inosine. The possible identity of this enzyme with Kalckar's nucleoside phosphorylase and the nature of the equilibrium involved are under investigation.

Early effects of testosterone propionate on the accessory sex glands of castrate rats. GUILFORD G. RUDOLPH AND GEORGE R. MENEELY (introduced by NORMAN S. OLSEN). *Radioisotope Unit, Veterans Administration Hosp. and Depts. of Biochemistry and Medicine, Vanderbilt Univ., Nashville, Tenn.*

Succinic and malic dehydrogenase activities and lactic acid concentration of the seminal vesicles and prostates of castrate rats, untreated and injected with testosterone propionate, and normal mature rats were measured. It was found that the activities of both enzyme systems were lower in the glands from castrate rats than from normal mature rats. Twenty hours after the injection of testosterone propionate into the castrate rats the level of succinic dehydrogenase activity in the seminal vesicles was about equal to that of the normal. The level in both glands at 48 and 72 hours after injection was higher than that found in the normal. The injection of the hormone resulted in an increased malic dehydrogenase activity up to the normal mature level after about 48 hours. The glands from the castrate rats produced about four times as much lactic acid (dry weight basis) as did the glands from the sexually mature rats. Following the injection of the hormone lactic acid production of the glands was similar to the untreated castrates.

Complex nature of the ribose-5-phosphate dehydrogenating system. HENRY Z. SABLE (introduced by C. F. CORI). *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo., and Dept. of Biochemistry and Nutrition, Tufts College Med. School, Boston, Mass.*

Reinvestigation of enzymes in yeast and rat liver, first described by Dickens, which catalyze electron transfer to TPN from added ribose-5-phosphate (R-5-P), shows that both DPN and TPN can be reduced by R-5-P. DPN reduction is due to formation of triose phosphate from R-5-P and is inhibited by poisoning triose-phosphate-dehydrogenase with iodoacetate. TPN reduction is unaffected by iodoacetate, cysteine, inorganic phosphate or Mg^{++}. TPN reduction in the presence of yeast or liver proteins occurs slowly at first (lag period) and then becomes more rapid. The lag is accentuated in a fraction of yeast maceration juice precipitated between 0.45 and 0.65 saturation with ammonium sulfate. On refractionation, the 0.52–0.59 saturated fraction shows a fourfold increase in the lag period. Preincubation of R-5-P with the 0.45–0.52 saturated fraction, itself unable to catalyze TPN reduction, caused a marked shortening of the lag observed with the 0.52–0.59 saturated fraction alone. Preincubation of R-5-P with the 0.52–0.59 or 0.45–0.65 fraction before addition of TPN completely abolished the lag. This evidence indicates that R-5-P is not the substrate of the TPN-linked dehydrogenase, but that a substance formed by a preceding anaerobic reaction is the oxidizable substrate. Certain other carbohydrates have been tested, and do not cause TPN reduction. The nature of the anaerobic phase and possible identity of the oxidizable substrate will be discussed.

Ultraviolet absorption spectra of peptides and amino acids. LEO J. SAIDEL AND A. ROBERT GOLDFARB (introduced by THEODORE WINNICK). *Dept. of Biochemistry, Chicago Med. School, Chicago, Ill.*

By making minor changes in the Beckman Model DU Spectrophotometer and by correcting for stray radiation, it is possible to use this instrument for the study of spectra of compounds in the ultraviolet region from 200 mμ to 230 mμ. Applied to proteins this technique has yielded interesting relationships which have been presented by Goldfarb, Saidel and Mosovich elsewhere. The present study of the spectra of peptides and amino acids has revealed striking quantitative differences between these two groups of compounds which must be caused by the peptide bond. Thus a valuable tool for the study of this biologically important bond is provided. Most of the common amino acids, with certain exceptions indicated below, have relatively low absorptions in this region. Thus at 205 mμ, which is a good working wave length, the molecular absorptivities of these amino acids are all below 150. A number of dipeptides of these amino acids have been studied. These all have a high molecular absorptivity at 205 mμ of about 3500. The aromatic amino acids, and histidine have a high maximum in this region. The S-containing amino acids and arginine have a relatively high absorption but no maximum. The tested dipeptides of the high absorbing amino acids have a much higher absorptivity than the sum of the constituent amino acid absorptivities. The tested tripeptides of low absorbing amino acids have an average absorptivity per peptide bond of about 2800. The earlier report on proteins presented comparable results on several proteins which ranged from 3000–3800.

Metabolism of acetone. WARWICK SAKAMI AND JEAN LAFAYE* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

C^{14} carbonyl-labeled acetone and C^{14} carboxyl- and carbonyl-labeled acetoacetate have been ad-

ministered to rats and the distribution of isotope in the liver glycogen determined. The acetone-C^{14} was incorporated into all of the glycogen carbons with significantly higher labeling in the 2,5 than in the 1,6-positions. The isotope introduced as acetoacetate was incorporated almost exclusively into the 3,4-carbons. The activities of the carbon fractions of the glycogen expressed in counts per minute per milligram of carbon were, in the acetone experiment, *1,6*:180, *2,5*:283, *3,4*:278, in the acetoacetate experiment *1,6*:22, *2,5*:26, *3,4*:1264. These results indicate that acetone is metabolized in part via some pathway other than carboxylation to acetoacetate (PLAUT AND LARDY). *J. Biol. Chem.* 186: 705, 1950) or a two- and one-carbon cleavage in which acetate and formate or metabolites of these compounds are formed (SAKAMI. *J. Biol. Chem.* 187: 369, 1950). The data do not determine whether an additional pathway of acetone metabolism is present or whether the manner in which 'formate' is formed from acetone has been incorrectly formulated. Of particular interest is the possibility of a direct conversion of acetone to a three carbon intermediate of glycolysis in that such a mechanism could provide for a net formation of carbohydrate from fatty acids. The results of our experiment are consonant with this mechanism.

Effects of beta-propiolactone on blood components. BENJAMIN E. SANDERS AND MADELAINE MAYKUT (introduced by GEORGE H. MANGUN). *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

Studies in this laboratory have shown beta-propiolactone (Betaprone) to be an active virucide and bactericide in the presence of blood and plasma. Gresham *et al.* (1948) and Long and Purchase (1950) have presented a series of papers on reactions of beta-propiolactone in aqueous solution. Little effect is observed on a number of plasma constituents studied. A moderate increase in erythrocyte fragility occurs in hypotonic saline when whole blood is treated with 1500 gm/l. of Betaprone. Erythrocyte potassium loss from stored blood differs only slightly from the controls. Glucose disappearance is markedly retarded after addition of Betaprone to whole blood. However, this has been shown to be a consequence of acid formed, since with adjustment of pH glycolysis again proceeds at the normal rate. The clotting rate of fibrinogen with preformed thrombin is unaltered at concentrations up to 2000 mg/l., although low recoveries of fibrinogen were observed at concentrations above 1500 mg/l. The Quick one stage prothrombin time is prolonged indicating that either prothrombin or accelerator globulin is affected. Betaprone was shown to have no significant effect on complement activity nor on *Br. abortus* and syphilis antibodies by complement fixation. Investigations are continuing on a number of other blood components.

Quinolinic acid excretion and metabolism in man. HERBERT P. SARETT. *Nutrition Research Lab., Tulane Univ. School of Medicine, New Orleans, La.*

Quinolinic acid has been measured in urine by microbiological assay of the nicotinic acid formed following decarboxylation. Autoclaving quinolinic acid at 121° for one hour in 1N sulfuric acid solution yields 21–26% (23.4%) of the theoretical amount of nicotinic acid, whereas in the presence of 50% acetic acid 80–83% (81.6%) conversion is obtained. Twenty-two subjects on varied diets excreted 2.9–12.9 mg. of quinolinic acid per day (average 6.4 mg.) while 23 subjects who were maintained on basal diets low in tryptophan and protein excreted 2.3–8.5 mg. per day (average 4.6 mg.). The extra excretion of quinolinic acid following several daily supplements of 5 gm. DL-tryptophan to each of 11 subjects varied from 2.3–21 mg. per day, and averaged 9.3 mg. Administration of 1 and 2 gm. of quinolinic acid daily to 2 subjects led to increased excretion of N^1-methylnicotinamide and of the 6-pyridone of N^1-methylnicotinamide. In one subject the extra excretion of these compounds following 2 gm. of quinolinic acid daily was equivalent to that obtained after administration of 1 gm. L-tryptophan daily. In the second subject quinolinic acid was much less effective than this. In both subjects the extra quinolinic acid which was excreted in the urine accounted for only 1.5–2.4% of the quinolinic acid administered.

Studies on the synthesis of hippuric acid. N. SARKAR,* M. FULD* AND D. E. GREEN. *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

Cohen and McGilvery have carefully studied hippuric synthetase in washed residues of rat liver. In confirmation of Leuthardt and Schneider, this condensing system has been localized in mitochondria, and shown to be part of the cyclophorase complex of rabbit liver and kidney. Dinitrophenol abolishes hippuric synthetase activity. Benzoic acid can be replaced by other heterocyclic carboxylic acids, e.g. furoic acid, cyclohexane carboxylic acid, nicotinic acid, and quinaldinic acid, and by aliphatic acids such as α-hydroxy isobutyric acid. Glycine cannot be replaced by either β-alanine or taurine. Glycine does not condense with phenylacetic acid or cholic acid under the same conditions suitable for condensation with benzoic acid. In the liver cyclophorase system, the relatively rapid conversion of both serine and glutathione to glycine can be shown by means of hippuric acid formation. Some studies on other synthetic reactions carried out in this system will be reported on.

Effect of nitrogen fertilization upon the biological value and amino acid content of corn. H. E. SAUBERLICH,* WAN-YUIN CHANG* AND W. D. SALMON. *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.*

The nitrogen content of corn kernels from a series of different varieties ranged from 1.09%–1.41% when grown with low nitrogen fertilization, but was significantly increased when grown with adequate nitrogen fertilization (ranged from 1.52%–1.92%). Growth experiments with rats demonstrated that the low-N samples were very inferior to the high-N samples when used to supplement low protein diets or as the sole source of protein in the diet. For example, when diets containing 95% of corn as the only source of protein were supplemented with tryptophan and lysine, moderately good growth was obtained with the high-N corn (20–25 gm/week), whereas growth was very inferior with the low-N corn (often only 1–2 gm/week). To obtain growth comparable to that of the high-N corn, additional supplements of isoleucine, threonine, and valine were necessary. The main effect of nitrogen fertilization appeared to be on the quantity of protein in the corn rather than on the quality. Microbiological assays for 18 amino acids confirmed the above results. The amounts of the individual amino acids varied with the nitrogen content of the corn. The amino acids were present in the high-N corn samples in amounts ranging from 25%–100% higher than the values obtained for the low-N corn. Values for the individual amino acids as well as growth data will be presented.

Acetate oxidation by *Micrococcus lysodeikticus*. HOWARD J. SAZ* AND L. O. KRAMPITZ. *Dept. of Microbiology, School of Medicine, Western Reserve Univ, Cleveland, Ohio.*

The failure of suspected carrier intermediates to attain the same specific activity as the respiratory carbon dioxide during the oxidation of labeled acetate has been the major problem in the elucidation of the mechanism of acetate oxidation by bacteria. Oxidation of $C^{14}H_3COONa$ by lysed preparations of *Micrococcus lysodeikticus* has been accordingly conducted in the following manner: the quantity of acetate required to approximately half saturate a given amount of enzyme for maximal oxidative activity was determined. The amount of enzyme chosen was that which would permit isolation of a sufficient quantity of the possible intermediates for degradation and specific activity determination, should they exist as enzyme-substrate complexes. After ten minutes the oxidation was stopped and the acid-soluble constituents of the enzyme preparations were extracted. This material was chromatographed on columns which separate mono and dicarboxylic acids. Seven acid constituents were obtained in varying amounts and specific activities. An endogenous control, treated in the same manner contained the same constituents but in lower concentrations. The specific activities of the acetate and succinate were determined. If endogenous corrections are not applied the results indicate almost complete isotopic equilibration of the recovered acetate with the isolated succinate. Similarly several of the other unidentified acids show complete equilibration with the acetate (milliequivalent basis). These results cast some doubt on conclusions drawn from experiments on bacterial oxidations in which there was non-equilibration of carriers with substrates. Further results will be reported on the mechanism of acetate oxidation in *M. lysodeikticus*.

Intracellular distribution of arginase activity. ARNOLD H. SCHEIN AND EMILY M. YOUNG (introduced by HAROLD B. PIERCE). *Dept. of Biochemistry, College of Medicine, Univ. of Vermont and State Agricultural College, Burlington.*

Differential centrifugation was employed to separate aqueous homogenates of rat liver into four fractions: *1*) a nuclear fraction which consisted of free nuclei, free mitochondria and unbroken cells, *2*) mitochondria, *3*) microsomes, *4*) 'supernatant fluid', containing very fine particles and soluble material of the cell. The homogenization procedure and conditions of centrifuging were similar to those previously described for the intracellular localization of uricase activity in which typical nucleic acid and succinoxidase distributions were obtained. (*Federation Proc.* 9: 224, 1950). The arginase activities of the homogenates and cell fractions were measured essentially as described by Van Slyke and Archibald. Arginase was found to be ubiquitously distributed in the four fractions. The nuclear and mitochondrial fractions each possessed 30–35%, while the microsomes contained 17% and the supernatant fluid, 12% of the total homogenate activity. The ratios of the specific activities (μg. urea produced/μg. N/10 minutes) of the fractions to that of the homogenate were: nuclear fraction 2, mitochondria 1, microsomes 1 and supernatant fluid about 0.3. Repeated washings (seven times) did not diminish the specific activity of the mitochondria, suggesting that in this fraction the enzyme and particulate matter form an intimate association.

Transamination as a step in tyrosine metabolism. BERNARD SCHEPARTZ (introduced by JAMES H. JONES). *Dept. of Biochemistry, Jefferson Med. College, Philadelphia, Pa.*

Previous investigators have reported that the oxidation of tyrosine in minces or extracts of liver is accompanied by the utilization of four atoms of oxygen per molecule of tyrosine catabolized, and

that no free ammonia is liberated. Since the products of tyrosine oxidation in liver are acetoacetate and, probably, fumarate and a molecule of carbon dioxide, it would appear that five atoms of oxygen should be required, on the assumption that the nitrogen is removed by oxidative deamination. If the first step were a transamination, both the oxygen uptake and the absence of ammonia could be explained. This possibility has been investigated in buffered extracts of an acetone-powder of liver. The system had no significant effect on tyrosine unless supplemented with ketoglutarate, in which case the oxygen uptake was greatly increased, tyrosine disappeared, and acetoacetate and glutamate were formed. Approximately four atoms of oxygen were utilized for each molecule of tyrosine catabolized, and no free ammonia was produced. These results suggest the hypothesis that tyrosine, in the course of its normal metabolism in liver, undergoes transamination rather than oxidative deamination as the initial step in its conversion to ketone bodies. (This investigation was supported in part by a research grant from the Natl. Insts. of Health, Public Health Service.)

Accumulation of glycerylphosphorylcholine during incubation of minced rat intestines. GERHARD SCHMIDT, LISA HECHT* AND S. J. THANNHAUSER. *Boston Dispensary, Tufts Med. School, Boston, Mass.*

It had been demonstrated (*Federation Proc.* 8: 249, 1949) that glycerylphosphorylcholine accounts for a considerable part of the acid soluble P fraction in fresh livers of beef, lamb, rabbit and —to a lesser extent—in fowl, and that this substance is the principle product of the enzymatic degradation of lecithin in beef pancreas. In fresh rat liver, however, the amounts of glycerylphosphorylcholine were negligible, and the enzymatic degradation of lecithin in rat intestine at pH ranges between 7 and 8 resulted in the almost complete hydrolysis to fatty acids, glycerol, choline and inorganic phosphate. It appeared as probable that an intermediary accumulation of glycerylphosphorylcholine in autolyzing intestinal mucosa was prevented because of its rapid hydrolysis by the potent phosphatases of this tissue. It could be demonstrated that glycerylphosphorylcholine was the predominant product of the enzymatic degradation of lecithin in intestinal mucosa when the incubation was carried out at pH 5.3 or in media of higher alkalinity in presence of 0.15 M glycerophosphate which strongly inhibited the phosphatases. It is concluded that glycerylphosphorylcholine is the principle intermediary of lecithin metabolism in rat intestines. This is of interest because of the rapid turnover of phospholipid phosphorus in this organ.

Isolation of adrenocortical compounds from normal male urine. JOHN J. SCHNEIDER. *Dept. of Exper. Med., Jefferson Med. College, Philadelphia, Pa.*

Using methods similar to those already described (SCHNEIDER. *J. Biol. Chem.* 183: 365, 1950) normal male urine has been further examined for compounds of the adrenocortical type. Of the crystalline compounds recovered, the chief again proved to be 17-hydroxy 11-dehydrocorticosterone which was isolated in a yield of 59 γ per liter. 17-hydroxycorticosterone was obtained in a pure state but in substantially smaller amounts. Other compounds isolated include pregnane-17 (α), 21-diol-3, 11, 20-trione, pregnane-3 (α), 17 (α), 21-triol-11, 20-dione and the previously described and now further characterized Cpd. II. Studies bearing on the stability and rearrangement of 17-hydroxy 11-dehydrocorticosterone will also be described.

Mechanism of hypoxanthine synthesis from glycine, formate and 4-amino, 5-imidazolecarboxamide. MARTIN P. SCHULMAN* AND JOHN M. BUCHANAN. *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

The existence of a ribotide of 4-amino, 5-imidazolecarboxamide as an important intermediate in purine synthesis is supported by two lines of evidence. 4-amino, 5-imidazolecarboxamide reacts with HCOOH approximately mole for mole to form hypoxanthine in pigeon liver extracts. To investigate the mechanism of this reaction, C^{14}-carboxamide was incubated for a short period with homogenates in the presence of non-isotopic inosinic acid and hypoxanthine or inosine. The specific activity of the isolated inosinate was considerably greater than that of the inosine or hypoxanthine, indicating that the elements of ribose and phosphate are added to the carboxamide prior to ring closure with HCOOH. Under similar experimental conditions, $NH_2CH_2C^{14}OOH$ is likewise incorporated into inosinate in greater concentration than into inosine or hypoxanthine. The other line of evidence is that the addition of inosinate to the extract stimulates the incorporation of $HC^{14}OOH$ into purine to a much greater extent than it does the incorporation of $NH_2CH_2C^{14}OOH$, indicating the existence of a mechanism for the incorporation of formate in addition to the *de novo* synthesis of hypoxanthine from glycine and formate. In addition, incubation of $HC^{14}OOH$ with inosinate results in labeling of the purine moiety almost exclusively in the 2 position. This suggests an equilibrium reaction in which inosinate cleaves to HCOOH and carboxamide ribotide, which then reacts with $HC^{14}OOH$ yielding inosinate labeled in the 2 position. Thus,

the carboxamide ribotide may be a key intermediate in the synthesis of purines from glycine and the carboxamide.

Genetic differences in the requirement for ribosenucleic acid and glycine in Drosophila melanogaster. JACK SCHULTZ AND MARGARET MANN SERVICE (introduced by T. F. LAVINE). *Dept. of Genetics and Cytochemistry, Inst. for Cancer Research and the Lankenau Hosp. Research Inst., Philadelphia, Pa.*

The nutritional requirements of the wild type Drosophila melanogaster are being compared with those of an isogenic group of seven mutants as part of a study of the relation of the nucleic acids to the genes. Aseptic growth of Drosophila occurs on a chemically defined medium (Schultz, St. Lawrence and Newmeyer, 1946), composed of amino acids as in casein, all the B vitamins, sucrose, salts and ribosenucleic acid (or adenylic acid). Omission of the nucleic acid from the medium slows the time of puparium formation, and decreases the weight of the adult fly. Only one of the seven stocks differed significantly from the wild type on this medium. With only the ten amino acids essential for the mammal plus glutamic acid, the omission of nucleic acid produced more drastic effects. Differences between the mutant stocks are now shown. On this nucleic acid-free medium glycine has a growth-promoting effect for the wild type, dependent on the amount of folic acid present. The present work confirms a relation between folic and nucleic acid requirements. Interrelations also were found between the requirements for methionine and those for folic and nucleic acid. The mutant stocks, tested on these media, showed characteristic differences in their requirements. Since the mutants chosen are distinguished by effects on morphological characters or pigment development, the diversity of their nutritional requirements shows the ramifying effects of single gene changes.

Properties of pyruvic oxidase. RICHARD S. SCHWEET, V. JAGANNATHAN AND BERNARD KATCHMAN (introduced by D. E. GREEN). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

A soluble pyruvic oxidase obtained from pigeon breast muscle has been reported by Jagannathan and Schweet (Abstracts A.C.S. Meeting, September, 1950). The enzyme was essentially inactive in the absence of cocarboxylase and showed slight activation with Mg ion. However, by a modified procedure the enzyme could be prepared in a form which was dependent on added Mg ion. Only Mn ion, of other metals tested, could substitute for Mg ion. Added DPN, TPN and coenzyme A had no effect on enzymatic activity, and were not detected in significant amounts in the purified enzyme. However, flavin compounds and 'protogen' were found to be present in these samples. Preparations were studied in the Tiselius electrophoresis apparatus and Spinco analytical ultracentrifuge in phosphate and barbiturate buffers of varying pH and ionic strengths. The homogeneity of the enzyme by these criteria will be discussed. Coenzyme A was required for the acetylation of sulfanilamide and p-aminoazobenzene by the pyruvate-pyruvic oxidase system coupled with the appropriate acceptor enzymes. ATP was not required and panthetine (LBF) was inactive in this system.

Probable maximum specific activity of macerans amylase. SIGMUND SCHWIMMER AND A. K. BALLS. *Corn Industries Research Fndn. Project, Enzyme Research Div., Bureau of Agricultural and Industrial Chemistry, U. S. Dept. of Agriculture, Albany, Calif.*

The concentrated culture fluid of *B. macerans* was fractionated by precipitations between 44 and 66 vol. % of ethanol at 25°, then further purified by precipitation from 44 vol. % ethanol at −10°. The macerans amylase was then adsorbed on raw starch, washed and eluted as described by Hale and Rawlins (*Cereal Chem.* In press, January 1951). The concentrated eluate showed an activity of 88 units/mg. of protein nitrogen. The protein was then precipitated with ammonium sulphate in 4 fractions at pH 8. The best of these fractions showed a specific activity of 140. By employing the method of calculation proposed by Falconer and Taylor (*Biochem. J.* 40: 835, 1946), a maximum value of 165 ± 15 for the specific activity of the enzyme was obtained. The relation between the change of color of a starch digest with iodine and the extent of hydrolysis is shown by Schwimmer and Balls (*J. Biol. Chem.* 179: 1063, 1949). Using these data, the rate of splitting by a preparation of the foregoing activity would be 1 'mEq. of glucosidic bonds'/min/mg. of protein nitrogen. This value is less than that observed for most pure enzymes, though of the same order of magnitude. It may well represent a minimum, however, on the assumption that, at least initially, the average length of the residual starch chains is longer (and therefore more chromogenic with iodine) during hydrolysis with macerans than with α-amylase.

Synthesis of glucose-6-phosphate and 6-phosphogluconate. J. E. SEEGMILLER (introduced by B. L. HORECKER). *Natl. Insts. of Health, Bethesda, Md.*

Available methods for synthesis of glucose-6-phosphate are time consuming and involved. Glucose-6-phosphate has now been synthesized by a relatively simple and quick procedure which yields

45-gram quantities of nearly pure material from a single laboratory-scale run. Glucose was incubated for 16 hours at 25° with polyphosphoric acid (tetraphosphoric acid, Monsanto). Excess inorganic phosphate was removed by crystallization of the sodium salt, and phosphate esters other than glucose-6-phosphate were destroyed by prolonged acid hydrolysis. Glucose-6-phosphate was collected and purified as the barium salt by precipitation with ethanol. The intermediate and final products were assayed spectrophotometrically by reduction of triphosphopyridine nucleotide (TPN) in the presence of *Zwischenferment* (glucose-6-phosphate dehydrogenase). The purity of the barium salt varied from 76–89% on the basis of dry weight and 83–93% on the basis of total organic phosphorus. Barium-6-phosphogluconate was prepared from barium glucose-6-phosphate by a modification of the method of Robison and King (*Biochem. J.* 25: 323, 1931). The time of oxidation with bromine was reduced from 48–2 hours, greatly improving the yield and purity of the final product. Spectrophotometric assay with purified phosphogluconic dehydrogenase and TPN showed the product to be 95% pure based on dry weight. Phosphogluconate accounted for 98% and glucose-6-phosphate for less than 0.5% of the total phosphorus.

Coagulation factors in ultraviolet irradiated normal human plasma. GEORGE Y. SHINOWARA. *Dept. of Pathology, Ohio State Univ., Columbus.*

Fibrinogen was determined as thrombin clottable protein in Fraction I (Cohn) obtained from 4 cc. of plasma; prothrombin, by the homologous isolation technic (*Am. J. Physiol.* 159: 303, 1949). Thromboplastic plasma component (TPC) was estimated by activation of nonclottable globulin of Fraction I in mixtures containing 0.0062 M calcium, 5 units prothrombin/cc., and 12 units thromboplastic cell component/cc.; the resulting thrombin was measured by clotting time determinations with fibrinogen. All the biochemical reagents were of human source and quantitatively standardized against Natl. Insts. of Health thrombin. American Red Cross dried normal human plasma, stored 4–6 years, was found to contain adequate amounts of coagulation factors: fibrinogen, 0.290% (*J. Lab. & Clin. Med.*, in press); prothrombin, 63.7 units/cc. (*J. Applied Physiol.* 2: 192, 1949); TPC, 43.9 units/cc. However, 8 samples exposed to ultraviolet emitting intense radiation of 253.7 and 184.9 mμ wavelengths gave the following results: fibrinogen, 0.319 (0.294–0.353) %; prothrombin, 53.2 (48.3–60.8) units/cc.; TPC, 6.4 (5.0–7.9) arbitrary units/cc. The latter indicated that maximum amounts of nonclottable globulin of Fraction I from irradiated plasma in activation mixtures only slightly converted prothrombin to thrombin. Therefore, the currently available plasma treated with ultraviolet radiation to destroy possible viral and bacterial contaminants would be of questionable value for a hemophiliac or any individual whose hemorrhage is due to TPC deficiency.

Origin of the carbon, nitrogen, and phosphorus of T_6 bacteriophage. M. S. H. SIDDIQI (introduced by FRANK W. PUTNAM). *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Escherichia coli was grown in a medium containing N^{15}, P^{32}, and carboxyl-C^{14} lysine. The bacteria were centrifuged down, washed, and resuspended in isotope-free medium and allowed to grow 10-fold to produce differentially labeled cells. These cells contained C^{14} only in lysine, and all three isotopes were in a higher concentration in nucleic acid and protein than in the acid soluble fraction. After multiple infection the single generation of virus was purified and the isotope distribution determined. Considerable amounts of all three tracers were found in the virus. The radioactive lysine in the phage amounted to only 1–2% of the total host C^{14} lysine but was more than twice the host acid soluble C^{14} lysine. The 14% of the virus lysine originating in the host must have come mainly from bacterial protein. The bacterial acid soluble fraction contained insufficient N^{15} (only one-third) and insufficient P^{32} (only one-eighth) to account for the host contribution to phage nucleic acid. 37% of the virus nucleic acid N was derived from the host and 25% of the virus P. These results agree with earlier findings that bacterial desoxyribonucleic acid is the source of the host contribution to virus nucleic acid. Most of the virus lysine and most of the virus protein are synthesized *de novo*, preformed lysine contributed by the host coming chiefly from bacterial protein.

In vitro incorporation of l-C^{14}-DL-alanine into proteins of rat-liver granular fractions. PHILIP SIEKEVITZ* AND PAUL C. ZAMECNIK. *Med. Labs. of Collis P. Huntington Memorial Hosp. of Harvard Univ., at Massachusetts General Hosp., Boston.*

Radioactive alanine is taken up into proteins of a 0.25 M sucrose-separated rat-liver fraction containing mitochondria and microsomes. Treatment of the protein with ninhydrin, NaOH, or thioglycolic acid releases very little radioactivity. All the radioactivity is released from hydrolyzed protein by ninhydrin. In absence of substrate the preparation does not respire but takes up a small constant amount of alanine. This uptake is increased 2-to 3-fold by aerobic oxidation of alpha-ketoglutarate or succinate, or by anaerobic oxidation by ferricyanide of alpha-ketoglutarate or malate. The complete system for alpha-ketoglu-

tarate oxidation, phosphorylation, and alanine uptake contains Mg^{++}, inorganic phosphate and adenylic acid. The optimal pH is 7.5. There is no increase in incorporation under anaerobic conditions, or in presence of dinitrophenol, azide, fluoride, or hexokinase. In absence of substrate, uptake is increased aerobically or anaerobically by adenosine triphosphate (ATP) if Mg^{++} is present, but not by adenylic acid. This activation by ATP is abolished by hexokinase, depressed by dinitrophenol, and not affected by azide or fluoride. Uptake of alanine and oxidative phosphorylation are both inactivated by incubation at 37° for 30 minutes but ATP still stimulates uptake after this treatment. A mitochondrial preparation nearly free of microsomes (cf. KELLER, E. B. *Federation Proc.* 10: 1951) does not incorporate alanine under conditions of active oxidative phosphorylation. Incorporation occurs only if microsomes, and not if supernatant or boiled microsomes, are added to mitochondria. After incorporation in a whole homogenate, relative specific activities were: microsomes, 15; mitochondria, 6; homogenate, 3; and supernatant, 1.

Nature of glutamic acid excreted in folic acid deficiency. MILTON SILVERMAN, HOWARD BAKERMAN* AND FLOYD S. DAFT. *Natl. Insts. of Health, Bethesda, Md.*

It has been established that folic acid deficiency, induced in the rat by succinyl-sulfathiazole, is accompanied by the excretion of excessive quantities of glutamic acid in the urine (BAKERMAN, ET AL. *J. Biol. Chem.* January 1951). An examination of the urine from such animals has shown that the excess glutamic acid is present in the form of an unidentified labile compound. Upon heating or alkali treatment the compound is converted to L-glutamic acid. Heating at 100°, for 60 minutes at pH 6.0–7.0, or treatment at pH 12 (KOH) for 2–3 hours at 23° results in maximal conversion to L-glutamic acid. Concentrates were prepared by passage of the urine through the cation exchanger, Dowex 50. Such concentrates show a 20-fold increase in glutamic acid activity on heating at pH 6.0.

In vitro studies on mechanisms involved in protein turnover. MELVIN V. SIMPSON (introduced by GERHARD SCHMIDT). *Dept. of Physiology, Tufts College Med. School, Boston, Mass.*

The breakdown phase of protein turnover has been investigated by labeling the proteins of the intact rat with S^{35}-methionine and subsequently studying its release from the proteins of liver slices. Total non-protein radioactive sulfur is determined after protein precipitation. The Krebs medium contains sufficient L-methionine to act as a trap, diluting the released isotopic methionine, thereby preventing its re-entry into protein. This permits the breakdown phase of protein turnover to be studied independently of the synthetic, as would not be the case were net breakdown determined. It has been found that conditions which inhibit energy production also inhibit release of labeled methionine from protein. Absence of oxygen or presence of cyanide inhibits approximately 85% of the release in a 4-hour incubation period. 2,4-dinitrophenol in concentrations insufficient to affect respiration in liver slices (1.0×10^{-4} M), inhibit about 50%. Higher concentrations (5.0×10^{-4} M) inhibit almost completely. These results are borne out when non-protein cystine is first removed before the digestion to sulfate, when non-protein methionine is isolated to constant specific activity by carrier techniques, and when the experiments were repeated using leucine-C^{14}-3. Similar results are obtained using different protein precipitants. Inhibition of respiration somewhat increases the amount of protein leached from the cell. The effect of this removal of protein is being evaluated. However, it appears at present that the release as well as the incorporation of amino acids during protein turnover is dependent upon an energy supply.

Effect of the adrenal cortical hormones and insulin on alanine metabolism. F. MAROTT SINEX (introduced by A. BAIRD HASTINGS). *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Experiments have been carried out to determine the effect of insulin and the adrenal cortical hormones on amino acid incorporation into protein, *in vitro*. Rat liver slices and diaphragm have been incubated in media containing alanine labeled in the carboxyl group with carbon-14. The effect of insulin and the adrenal cortical hormones on the incorporation of this alanine into tissue protein has been determined. Insulin, 0.6 units per ml. added to the incubation medium, increased the incorporation of the carbon-14 of DL-alanine into the protein of diaphragm about 60%. An inhibition in incorporation of 31% was observed when diaphragm was incubated with DL-alanine in the presence of cortisone. Liver slices prepared 2 hours after injecting a fed rat intraperitoneally with ACTH incorporated only half as much carbon-14 into their protein as did liver slices from control rats. Liver slices from rats fasted overnight also incorporated 47% less carbon-14 into their protein than control slices from fed animals.

Liver lipids in threonine and lysine deficiencies in the rat. S. A. SINGAL, S. J. HAZAN,* V. P. SYDENSTRICKER* AND JULIA M. LITTLEJOHN.* *Med. College of Georgia, Augusta.*

It has been previously reported that there is an accumulation of lipids in the livers of rats receiving suboptimal amounts of threonine (*Federation Proc.* 8: 251, 1949). Further studies indicate that lysine deficiency in the rat produces a similar change in liver lipids. Rats on a diet completely deficient in lysine show little change in liver lipids. Supplements of lysine from 0.35–0.57% produce a graded growth response accompanied by an increase of liver lipids to 15.1%. Supplementary lysine at a level of 0.78% produces a maximum growth response under our conditions and a drop of liver lipids to 6.0%. Large amounts of dietary fat or choline do not significantly alter these results. The liver lipids of threonine and lysine deficient rats have been analyzed for total phospholipids, monophosphatides, free and esterified cholesterol and unsaturation. Results indicate a significant reduction in total phospholipids and monophosphatides and an increase in esterified cholesterol. The iodine number of the total lipids tends to fall in the deficiency state. Blood phospholipids, free and esterified cholesterol are unchanged.

High molecular weight nucleoprotein found in cytoplasms of a variety of healthy plant leaves. S. J SINGER, LUTHER EGGMAN, JEAN M. CAMPBELL AND SAM G. WILDMAN (introduced by CARL NIEMANN). *Kerckhoff Labs. of Biology, California Inst. of Technology, Pasadena.*

Ultracentrifuge experiments have demonstrated the presence of a high molecular weight component in cytoplasmic extracts from the leaves of a variety of healthy plants. These extracts were prepared by colloid-milling of leaves essentially as described by Wildman and Bonner (*Arch. Biochem.* 14: 381, 1947). Plants investigated so far include several varieties of tobacco, tomato, spinach, pea, and xanthium, extracts from all of which exhibit very similar sedimentation characteristics. Two major fractions appear, the faster-moving of which has a sedimentation constant of about 20 S, is apparently homogeneous ultracentrifugally, and constitutes about 30% or more of the total protein in the extract. The slower-moving fraction is heterogeneous, and has not yet been studied in detail. Electrophoretically, the two fractions migrate largely under a single peak at pH = 7. With tobacco leaf extracts (I) the 20 S component (II) precipitates irreversibly at pH = 6.0 and below, but is stable ultracentrifugally from pH = 6.0 – 8.5. By quantity centrifugation of I at 110,000 *g* at 0 – 5°C. for 3 hours, II is thrown down, largely freed of slower-sedimenting proteins, along with about 10% of faster-sedimenting material. Such a preparation of II contains 0.5 –0.9% TCA precipitable P, and shows the presence of adenine, quanine, cytidylic, and uridylic acids. Warming to 30°C. for 2 hours, or $(NH_4)_2SO_4$ precipitation, releases some of the TCA precipitable P. Precise physical characterization of II awaits further knowledge of its chemical nature and methods of purification.

Acetoin synthesis in highly purified carboxylase preparations. THOMAS P. SINGER AND JACK PENSKY.* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

A highly active α-carboxylase from wheat germ was first described by Bartlett (Thesis, Univ. of Chicago, 1942). This enzyme can be readily purified 6000-fold by a combination of isoelectric precipitation, ethanol, and ammonium sulfate fractionation. The best preparations obtained show a constant activity of Q_{CO_2} = 40,000 at pH 6 and 30°C. The enzyme requires Mg^{++} and DPT for full activity; Mn^{++} is less effective. Juni (unpublished experiments) has observed that acetoin synthesis accompanies carboxylase activity in various yeast preparations. We find that acetoin synthesis from acetaldehyde alone or from acetaldehyde and pyruvate occurs in highly purified wheat germ carboxylase and accompanies carboxylase and accompanies carboxylase activity throughout the purification procedure. Without added acetaldehyde no significant acetoin synthesis is observed from pyruvate, since the acetaldehyde produced by carboxylase action does not meet the saturation requirements for rapid acetoin formation. The ratio of acetoin formed from acetaldehyde alone to that formed from acetaldehyde and pyruvate remains constant (about 0.25) throughout the purification. The DPT and Mg^{++} requirements as well as the pH optima for carboxylase action and acetoin synthesis appear to be identical. Carboxylase and acetoin-synthesizing activities decrease to the same extent upon aging. While acetoin formation follows zero-order kinetics, CO_2 formation decreases markedly with time, since acetaldehyde is strongly inhibitory to carboxylase. The question as to whether the same prltein may be responsible for these diverse activities is being investigated by physical measurement of the homogeneity of the enzyme and by differential inactivation studies.

Paper chromatographic studies on products of the enzymatic degradation of desoxyribonucleic acid. H. R. SKEGGS,* W. BAUMGARTEN,* J. SPIZIZEN* AND L. D. WRIGHT. *Med. Research Div., Sharp and Dohme, Inc., Glenolden, Pa.*

The degradation of various desoxyribonucleic acid (DNA) preparations by desoxyribonuclease (DNA ase) and alkaline phosphatase has been followed with paper chromatography. Using the two phase iso-amyl alcohol-KH_2PO_4 system of Carter (*J. Am. Chem. Soc.* 72: 1466, 1950) it has

been possible to demonstrate an intermediate phase in the degradation of polymerized DNA. calf thymus) by crystalline pancreatic DNA ase, Completely polymerized DNA has an Rf of 0, is acid precipitable and highly viscous. The first stage of depolymerization is characterized by a product with an Rf of 0.95 that is acid precipitable but not viscous. The final product of depolymerization has an Rf of 0.75–0.80 and is neither acid precipitable nor viscous. While completely polymerized DNA cannot be degraded by alkaline phosphatase both the first and final products of DNA ase are split by alkaline phosphatase to component nucleosides with the liberation of inorganic phosphate in a ratio of 4 moles of phosphorous per mole of DNA. The degree of depolymerization of DNA can be estimated either by paper chromatographic techniques or by determination of the amount of phosphate released by alkaline phosphatase.

In vitro demonstration of an antihistaminic action of cortisone. DURWOOD J. SMITH (introduced by WILLIAM S. MCCANN). *Dept. of Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

The effect of cortisone on the reactions of the isolated surviving carotid arteries of swine was studied using the angioplethysmokymographic technique of Smith and Syverton. These vessels constrict to epinephrine, acetylcholine and histamine. The arteries were perfused with Tyrode's solution for 2–3 hours and control reactions to epinephrine, acetylcholine and histamine recorded, then the perfusate was modified by the addition of cortisone (1:1,000,000 of the free alcohol) and the reactions to the same stimuli were recorded; the perfusate was then shifted to the initial solution and final control reactions were obtained. No changes in the constriction to epinephrine or acetylcholine were observed. The response to histamine became progressively less under cortisone, in some experiments diminishing to 10% of control levels. This antihistaminic effect of cortisone appeared to be potentiated by acetylcholine.

Purification of cytochrome oxidase. LUCILE SMITH (introduced by BRITTON CHANCE). *Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia, Pa.*

The solubilized cytochrome oxidase preparation previously reported (SMITH, L. AND E. STOTZ. *Federation Proc.* 9: 230, 1950) has been subjected to further purification procedures, including precipitation with acid and adsorption of calcium phosphate gel. The enzyme activity, as measured by the rate of oxidation of reduced cytochrome c, was found to be fifty to sixty times greater in the repurified preparation than that of the phosphate extract of pig heart which was the starting material in the preparation. Using a Michaelis constant of 9×10^{-5} M (SLATER. *Biochem. J.* 44: 305, 1949) for the phosphate extract, the calculated Qo_2 is 1620 at 20°C. The Michaelis constant for the repurified preparation is not the same as that for the phosphate extract, and the calculated Qo_2 is over 10,000 in 1.5×10^{-5} M ferrocytochrome c. The repurified preparation showed an absorption spectrum with sharp peaks at 605 and 444 mμ and a smaller peak at 520 in the reduced form. In freshly prepared samples the ratio of cytochrome oxidase activity to the change in optical density on reduction at 605 and 444 mμ is fairly constant, and there is a constant ratio of the optical density at 605 mμ to that at 444 mμ. On standing at 4° for several weeks, the cytochrome oxidase activity gradually decreases to zero, while there is no change in the absorption spectrum. Addition of cyanide to the preparation brings about a change in the absorption spectrum, both with the oxidized form and with the preparation reduced by hydrosulfite.

Glutathione synthesis from glutamyl cysteine and glycine. JOHN E. SNOKE AND FRANK ROTHMAN (introduced by KONRAD BLOCH). *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

The synthesis of glutathione from the three constituent amino acids by a soluble enzyme system obtained from pigeon liver has been described by Johnston and Bloch (*J. Biol. Chem.* January, 1951). Present investigations concerned with the mechanism of this process have revealed that the same enzyme preparations are capable of forming glutathione by the condensation of synthetic γ-L-glutamyl-L-cysteine and glycine. The rate of formation of glutathione from the dipeptide is approximately proportional to the amount of enzyme present. The synthesis of the peptide bond does not occur in the absence of adenosine triphosphate. Conditions have been found which lead to the inactivation of the enzyme system responsible for glutathione synthesis from the three amino acids, but do not effect the conversion of glutamyl cysteine to the tripeptide. Such conditions are precipitation with ethanol and incubation of the enzyme system at 37°. The synthesis of glutathione from glutamyl cysteine and glycine is stimulated by a heat stable cofactor, probably identical with that required for glutathione synthesis from the three amino acids.

Chromatographic analysis of protein mixtures on a cation exchange resin. HERBERT A. SOBER AND GERSON KEGELES (introduced by JESSE P. GREENSTEIN). *National Cancer Inst., Natl. Insts. of Health, Bethesda, Md.*

The frontal analysis method of Tiselius and Claesson has been applied to artificial mixtures of purified proteins using a cation exchange resin as the adsorbent material. The effluent from the column was examined continuously by refractometric methods. Mixtures containing varying proportions of bovine plasma albumin (BPA) and human carboxyhemoglobin (CoHb) were investigated. Quantitative recoveries have been obtained. A systematic investigation has demonstrated that pH, ionic strength, resin form, protein concentration etc. affect the resolving power of the absorbent and the data obtained permit the construction of absorption isotherms. Resolution of BPA and CoHb has been obtained on the ammonium form of the cation exchange resin with the proteins in 0.01, 0.05 and 0.1 ionic strength sodium buffers in the pH range of 4.5–8.6. However, prior treatment of the ammonium salt form of the resin with sodium buffer, limits resolution of BPA and CoHb to a pH range of 6.0–7.5. When the cation in the buffered protein is identical with that in the base used for the final cycle of the resin, little or no protein separation is achieved. There is, moreover, a specific effect on the resolution of proteins of a variation in the cation of the protein solution, even when this cation differs from that on the resin. As a result of these studies, a tentative mechanism is postulated, which involves cation exchange as an essential prerequisite in the chromatographic analyses of these protein mixtures.

Determination of acetone, acetoacetate, and β-hydroxybutyrate. WARREN M. SPERRY AND FLORENCE C. BRAND.* *Dept. of Biochemistry, New York State Psychiatric Inst., New York City.*

The method of Greenberg and Lester (*J. Biol. Chem.* 154: 177, 1944) has been studied in detail and modified at several points. A simple procedure by which acetone is distilled quantitatively into the 2,4-dinitrophenylhydrazine reagent permits the separate, direct determination of acetone plus acetoacetate and of β-hydroxybutyrate in the same sample and avoids the large loss of acetoacetate which may occur during the chromate oxidation. Conditions under which a sufficiently stable color can be developed in alkaline solution were found, and the sensitivity of the method was thereby considerably increased. With these and several other minor changes the method gives satisfactory results in the determination of acetone, acetoacetate, and β-hydroxybutyrate in pure solutions, but it has proved impossible to apply it to the analysis of blood. Large losses of acetoacetate, added to blood as the sodium salt, occur during deproteinization and also during desaccharification by procedures which are compatible with the method. Although similar losses of β-hydroxybutyrate, added to the blood as the calcium-zinc salt, did not appear to occur during deproteinization and desaccharification, the results obtained in the analysis of such filtrates were so erratic as to be useless; large differences between duplicate determinations were usually obtained.

Ultraspectrophotometrical studies of mouse tumors treated with colloid Au^{198}. M. SPIEGEL-ADOLF AND R. ROBBINS.* *Depts. of Colloid Chemistry and Radiology, Temple Univ. Med. School, Philadelphia, Pa.*

In continuation of former studies (*Federation Proc.* 9: 398, 1950) spontaneous mammary carcinomas of hybrid mice were infiltrated with 1 ml. of colloid Au^{198} of various concentrations. The solutions were made to contain either 110 μc. or 1.75 mc/1 ml. at the time of infiltration. Thereafter at various intervals ranging from 1–83 days the 31 treated mice died or were sacrificed. Control mice were kept for comparable times. Some radioautographs were made from the tumor and internal organs of mice sacrificed 1–2 days after Au^{198} infiltration. In nearly all cases a part of the primary tumor was extracted as described before (*Arch. Path.* 41: 307, 1947) and ultraspectrographed. Differential phosphorus determinations were made in 7 cases. Another part of the tumor and slices of inner organs were used for histological examinations. Radioautography showed that even 2 days after the infiltration the tumor contains twenty times as much radioactive material as the liver. Only the tumors infiltrated with 1.75 mc. seem to show a regular response. In the majority of cases (9 out of 12), the E_{265} values of the tumor extracts show a marked drop between 3–35 days after treatment. In the case of multiple tumors the not treated remain unchanged. The histological examination confirms the spectrophotometrical results in as much as the tumor tissue shows all states of regression and degeneration, the extent of which shows a satisfactory parallelism with the optical findings. The control mice confirmed former observations.

Effects of radon radiation upon mouse tumors. M. SPIEGEL-ADOLF AND R. ROBBINS.* *Depts. of Colloid Chemistry and Radiology, Temple Univ. Med. School, Philadelphia, Pa.*

In order to study the direct effect of radiation upon tumor tissue radon seeds (½ and 1 mc.) were implanted into mammary carcinoma of 21 hybrid mice; 9 comparable mice served as controls. The survival time of the implanted mice varied from 2–47 days. In practically all cases the tumor extracts (*Arch. Path.* 41: 307, 1947) were studied chemically and ultraspectrophotometrically. These investigations were supplemented by histo-

logical studies which included a number of inner organs. The smaller radon dosage uniformly lowered the absorption coefficient E_{265}, the average value in treated animals being 1.52, in untreated 2.32. Histologically various degrees of degeneration could be observed. In the group which had received the higher radon dosage the changes were uniformly much more spectacular. After an interval of 4 days, one tumor had completely disappeared, 8 tumor extracts showed absorption graphs characteristic for proteins, 1 extract showed no absorption peak, another a very low value only. The histological examinations revealed all degrees of regression in the tumor tissue up to complete necrosis. Contrary to some observations on mice treated with Au^{198}, kidney and liver did not show any major changes in radon implanted animals. Neither was an increase of lung metastasis observed. Microchemical total P (BERENBLUM AND CHAIN. *Biochem. J.* 32: 295, 1938) and lipoid P determinations in the tumor extracts showed that their P content is more than sufficient to account for the assumption that the absorption peak of the spectrophotometrical graph is due to nucleic acids.

Demonstration of the presence of Krebs cycle enzymes in *Drosophila melanogaster*. M. A. SPIRTES (introduced by SIDNEY WEINHOUSE). *Dept. of Genetics and Cytochemistry, Inst. for Cancer Research and Lankenau Hosp. Research Inst., Philadelphia, Pa.*

As part of a program for the study of chemical genetics in the fly *Drosophila*, the enzymatic activities of the mature larvae have been investigated in vitro. Since the Krebs cycle has been found in most organisms in which its presence has been sought the enzymes involved were chosen as the first to be studied. Unwashed homogenates, water extracts and acetone powder extracts have been tested by spectrophotometric and Warburg techniques. Activity was found for the following key enzymes of the cycle: *1*) aconitase, *2*) isocitric dehydrogenase, *3*) succinic dehydrogenase, *4*) fumarase, *5*) malic dehydrogenase and *6*) condensing enzyme. Direct analysis of boiled homogenates showed the presence of DPN in amounts of approximately 100 μg/gm. wet weight and 500 μg. of citrate/gm. wet weight. Oxygen was consumed in the presence of succinate using washed homogenates in about half the attempts made, but pyruvate required the addition of large amounts of DPN and Warburg's new yellow enzyme (*Biochem. Ztschr.* 266: 377, 1933) together with catalytic amounts of fumarate and Mg^{++}. Oxidation was enhanced somewhat by the addition of ATP and cytochrome C. Other enzymes peripherally connected with the cycle, namely cytochrome oxidase, oxalacetic decarboxylase and lactic dehydrogenase were also present. Thus the enzymatic structure of this insect so far resembles that of other animals studied, as expected from the similarity in nutritional requirements (SCHULTZ AND RUDKIN. *Proc. VIII Internat. Cong. Genetics, Hereditas Suppl.* 1949: 657).

Hydrolysis of dextran. HOMER E. STAVELY AND E. E. TOOPS, JR. (introduced by A. WILHELMI). *Pharmaceutical Research Div., Commercial Solvents Corp., Terre Haute, Ind.*

Dextran, a polysaccharide produced by *L. mesenteroides*, has been used in recent years as a plasma volume extender in shock, burns, and related conditions. For intravenous use the molecular size of dextran must be reduced. The acid hydrolysis of dextran has been followed by measurements of viscosity, aldehyde end groups and average molecular weights by the light scattering method. Dextran hydrolysates have been fractionated and the effects on rats and mice correlated with molecular size. Previous workers have established that dextran is made up of α-D-glucose linked glycosidically through the 1–6 positions, with side chains of unknown length linked through position 4 of some of the glucose units in the main chain. This can be most readily shown by periodate oxidation, (JEANES AND WILHAM. *J. Am. Chem. Soc.* 72: 2655, 1950) which yields $(1-\alpha)$ moles of formic acid per anhydroglucose unit; α = the fraction of the anhydroglucose units having a side chain linked through the 4 position. Periodate oxidations have been carried out on both native and partially hydrolyzed dextran, and the results show that the 1–4 linkage is hydrolyzed more rapidly than the 1–6 linkage, as expected. A precise method for measuring formic acid in polysaccharide oxidation mixtures will be described.

Disappearance from mouse tissues of carbon from assimilated sucrose. ROBERT STEELE (introduced by J. R. KLEIN). *Biology Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Ten male mice, C57 blacks, $3\frac{1}{2}$ to $6\frac{1}{2}$ months old, were each fed a single dose of uniformly enriched C^{14} sucrose. Total sucrose fed was 5.0 to 8.3 mg/gm. mouse body weight. Stock mouse pellet diet preceded and followed the dose. At various times, from $3\frac{1}{2}$ hours to 36 days, after C^{14} sucrose ingestion mice were killed by withdrawing heart blood under Nembutal anaesthesia and dissected immediately. Dissected parts were powdered at liquid nitrogen temperature and portions subjected to combustion. The resulting CO_2 was counted as $BaCO_3$. Specific activity was expressed in mμc/mg. tissue carbon for 1 μc.C^{14} fed/gm. mouse body weight. For the carbon of each of 31 organs or tissues, the logarithm of the specific activity was plotted against elapsed time. Each

curve was resolved into as many straight line components as necessary for its description. Organs concerned with alimentation required four component description, most other organs required three. The organic carbon of the solid portion of long bone has a component with a half-life of about 200 days. The 3½-hour concentration of this component is about 0.05 mμc/mg. carbon for 1 μc.C^{14} sucrose fed/gm. mouse body weight. Abdominal storage fat has a very low turnover rate as measured by this method. Throughout the 36 days its C^{14} concentration remained around 0.12 mμc/mg. carbon for 1 μc,C^{14} sucrose fed/gm. mouse body weight. (Research sponsored by Atomic Energy Commission.)

Influence of thrombin on platelet activity in blood coagulation. MARIO STEFANINI (introduced by WILLIAM H. FISHMAN). *Ziskind Labs., J. H. Pratt and New England Center Hosps. and Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Platelet suspensions were prepared in the cold by a differential centrifugation method from blood decalcified with Amberlite IR-100. After repeated washings with phosphate buffered saline (pH 7.3) platelets were suspended in defibrinated native serum. Thrombin, 5–50 units in 0.1 ml. volume of buffered saline, was added to 0.9 ml. of platelet suspension in test tubes kept at 37°C. Silicone coated glassware and needles were used throughout. The number of platelets, counted by direct method, decreased sharply and promptly in the samples containing thrombin, due to macroscopic clumping. Native plasma and plasma decalcified with Amberlite were variously depleted of platelets by centrifugation. Thrombin, 5–50 units in 0.1 ml. volume, was added to 0.9 ml. of each plasma preparation. Accelerator effect and prothrombin activity of serum were determined for each sample. It was observed that: *a*) prothrombin activity of serum was directly related to the number of available platelets, in native but not in Amberlite decalcified plasma; *b*) development of accelerator effect in serum was independent of the nature of the plasma and, to some extent, of the number of available platelets. It is concluded that: *1*) thrombin causes aggregation and clumping of platelets; *2*) when acted upon by thrombin, platelets probably release a factor (possibly Quick's thromboplastinoganase) which further accelerates the formation of thrombin. Presence of calcium is indispensable for the occurrence of this sequence of reactions, one of the autocatalytic mechanisms in blood coagulation.

Role of folacine and vitamin B_{12} in synthesis and utilization of choline by the rat as studied with C-14-glycine, formate and methionine. JAKOB A. STEKOL, KATHRYN W. WEISS* AND SIDNEY WEISS.* *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

Rats deficient in either folacine, B_{12}, or in both, were used. The synthesis of carcass choline and cysteine in deficient animals was compared with that by normal animals 3 hours, 20 hours and 48 hours after the injection of uniform amounts of C-14-formate, 2-C-14-glycine, or methionine with C-14 in the methyl group. The folacine or B_{12} deficient rat utilizes the formate or the glycine for choline and cysteine synthesis to a much smaller extent and at a much slower rate than the normal rat, the rate of uptake of C-14 into the methyl group of choline being smaller than the uptake into the ethanolamine moiety, particularly in the B_{12}-deficient rat. While in normal rat the C-14 concentration in the methyl group and in the ethanolamine moiety of choline decreases with time after injection of either formate or glycine, in the deficient rat there was steady increase in C-14 concentration which at no time, however, reached the concentration of C-14 in choline of normal rat. Homogenates of livers of B_{12} or doubly deficient rats had a much lowered capacity for the synthesis of methionine from either choline or betaine and homocysteine. Folacine and doubly deficient rat liver homogenates showed a greatly reduced ability to synthesize cysteine from homocysteine and serine presumably via cystathionine. The cleavage of the latter to cysteine by the liver preparations was not affected by either deficiency. Addition of B_{12}, folacine, or Citrovorum Factor to deficient homogenates did not alter the results. The carbon of the methyl group of methionine appears in the methyl group and in the ethanolamine moiety of choline via serine, presumably. The concentration of C-14 in each moiety of choline depends on time elapsed after the injection of radiomethionine and on the presence or absence of B_{12} and folacine in the diet.

Possible metabolic role of KCN in the rat as studied with C-14-KCN. JAKOB A. STEKOL AND SIDNEY WEISS.* *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

C-14-KCN was injected intraperitoneally into adult Wistar rats, and 24 hours later the radioactivity was determined in the whole blood, liver and carcass proteins, and in choline (isolated as the reineckate) and creatine (isolated as creatinine-potassium-picrate. All of these tissue components contained appreciable radioactivity. Furthermore, C-14-KCN administered together with bromobenzene to rats was utilized for the synthesis of cysteine and acetic acid, as was revealed by the radioactivity located in the cysteine and in the acetic acid of the *p*-bromophenylmercapturic acid which was isolated from the urine.

Aside from the well known metabolic conversion of cyanide to thiocyanate, these observations suggest possible formation and utilization of cyanide *in vivo* either per se or via formaldehyde-formate. Furthermore, the property of cyanides to reduce disulfides to the corresponding thionitriles, and the fact that vitamin B_{12} contains easily removable cyanide group, point to an interesting possibility of vitamin B_{12} as the 'carrier' of the metabolically elaborated cyanide with its attendant hematopoietic and other physiological properties.

Enzymatic synthesis and breakdown of citric acid. JOSEPH R. STERN,* BENJAMIN SHAPIRO,* EARL R. STADTMAN* AND SEVERO OCHOA. *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

Previous work (*Nature, London* 166: 403, 1950) suggested that crystalline condensing enzyme catalyzes the reversible reaction 1 (acetyl-coenzyme A + oxalacetate $\rightleftharpoons$ citrate + coenzyme A). This reaction can be coupled with reaction 2 (*l*-malate + $DPN_{ox} \rightleftharpoons$ oxalacetate + DPN_{red}), catalyzed by malic dehydrogenase, to give reaction 3 (acetyl-Co_{ox} + 1-malate + $DPN_{red} \rightleftharpoons$ citrate + Co A + DPN A). Reaction 3 can be followed spectrophotometrically, at $\lambda = 340$ mμ, from right to left. It proceeds to a small extent, an equilibrium being attained. The rate and extent of the reaction depend on the amount of Co A added. On further addition of transacetylase (*Federation Proc.* 9: 233, 1950), and in presence of orthophosphate, acetyl phosphate is formed through reaction 4 (acetyl phosphate + Co A $\rightleftharpoons$ acetyl-Co A + phosphate). The over-all reaction, with condensing enzyme, malic dehydrogenase, and transacetylase, is reaction 5 (acetyl phosphate + *l*-malate + $DPN_{ox} \rightleftharpoons$ citrate + phosphate + DPN_{red}). Reaction 5 is readily reversible but its equilibrium markedly favors citrate synthesis. Through reaction 1 citrate acts as an acetyl donor not only to orthophosphate, but also to sulfanilamide and choline in presence of suitable acetyl acceptor systems. The choline acetylation experiments were jointly carried out with S. R. Korey and D. Nachmansohn. These experiments indicated that choline acetylase catalyzes the reaction acetyl-Co A + choline $\rightarrow$ acetyl choline + Co A.

Glucose production and oxidation in normal and diabetic rats. DEWITT STETTEN, JR., ISAAC D. WELT* AND DWIGHT J. INGLE.* *Div. of Nutrition and Physiology, Public Health Research Inst. of the City of New York, Inc., and Research Labs., The Upjohn Company, New York City.*

Normal, phlorhizinized and alloxan-diabetic rats, under cyclopal anesthesia, were infused intravenously at constant rate with a solution containing sufficient C^{14}-glucose to insure glucosuria. Glucose was isolated as its phenylosazone from hourly urine samples, expired CO_2 was collected, and glycogen recovered from the livers at the end of the experiment. After the specific radioactivity of the excreted glucose had attained a plateau, its value was compared with that of the injected glucose. This ratio together with the known rate of injection permitted the calculation of the rate at which each animal was generating glucose from non-isotopic precursors. These rates proved to be 33.8 ± 4.30 mg/rat/hr. in the normal, 31.0 ± 1.83 in the phlorhizinized and 43.3 ± 1.83 in the alloxanized rat. There would thus appear to be a statistically significant overproduction of glucose in the alloxanized preparations of about 10 mg/rat/hr. From the specific activities of the exhaled CO_2 samples, it has been calculated that 23, 7 and 9% of the CO_2 was derived directly from blood glucose in the normal, phlorhizinized and alloxanized rats respectively. A marked diminution in the oxidation of glucose incident to alloxan or phlorhizin administration is thus indicated.

Ornithine metabolism. MARJORIE R. STETTEN. *Div. of Nutrition and Physiology, Public Health Research Inst. of the City of New York, Inc.*

The metabolic fate of the α- and the δ-amino nitrogens of ornithine has been studied by parallel feedings to mice for 9 days of samples of *dl*-ornithine labeled with N^{15} uniquely in the α or in the δ position. Much of the unnatural isomer was excreted in the urine. Similar contributions were made by each of the two amino groups to tissue protein N, non-protein N, amide N, urinary N and urea. About 6.5% of the tissue arginine was found in both cases to have arisen from dietary ornithine in 9 days. Only an insignificant trace of N^{15} was found in the α-amino group of tissue arginine when δ-N^{15} ornithine was fed and in the δ-amino group when α-N^{15} ornithine was given. The α-amino group of ornithine contributed extensively to proline and hydroxyproline and to a lesser extent to glutamic and aspartic acids. The δ-amino group contributed more extensively to glutamic acid than to proline. These results are taken as an indication that the most important initial step in the breakdown of ornithine involves the preferential loss of the δ-N to the pool of labile amino groups, giving an intermediate, presumably glutamic semialdehyde, which on cyclization and reduction is converted to proline.

Mutagenic activity of compounds related to mustard gas. CARL M. STEVENS AND AUGUSTA MYLROIE (introduced by C. S. VESTLING). *Fulmer Chemical Lab., State College of Washington, Pullman.*

As part of a study of the mechanism of the

mutagenic action of mustard gases, we have investigated the relation of chemical structure to activity in several types of related compounds. Using mutant strains of *Neurospora crassa*, the capacity of the compounds to produce backmutations was tested by the method described by Dickey *et al.* (*Proc. Nat. Acad. Sc.* 35: 581, 1949). Under the conditions of the test, monofunctional *beta*-chloroethyl sulfides ($RSCH_2CH_2Cl$) were found to be highly active, while the series of *beta*-chloroethyl amines ($R_2NCH_2CH_2Cl$) varied greatly in activity. Evidence was obtained, in confirmation of previous work, that different genes differed in susceptibility to the mustard-type compounds. In view of the pronounced vesicant activity of *beta*-chloroethyl sulfones and vinyl sulfones, representative compounds of this type were tested, but no mutagenic activity was found. The sulfonium compound $(HOCH_2CH_2)_2\overset{+}{S}CH_2CH_2SCH_2CH_2\overset{+}{S}(CH_2CH_2OH)_2$ was found to be an effective mutagen. The activity of this and other sulfonium compounds may provide an explanation for the observed mutagenic activity of 'aged' solutions of mustard-type compounds.

Microbiological assay for β-alanine. CHARLES J. STEWART, PHILLIP E. LEVEQUE AND TSOO E. KING (introduced by VERNON H. CHELDELIN). *Dept. of Chemistry, Oregon State College, Corvallis.*

Rapid analytical methods for pantothenic acid are limited to those employing microorganisms. However, even in improved extraction procedures the yields of pantothenic acid obtained are lower than those observed in rat tests. Further study of extraction procedures for the vitamin, as well as measurement of the constituent moieties of several pantothenic acid conjugates, would be aided by an adequate assay method for β-alanine. β-Alanine has been determined by yeast assay. However, in the presence of β-alanine the growth of yeast is strongly influenced by many amino acids, especially glutamic acid and cystine. Attention was therefore directed toward other assay organisms. A non-pathogenic strain of corynebacteria (*Corynebacterium xerose*) was observed to utilize β-alanine. This organism, like the panthogenic strains described by Mueller (1937), reaches half-maximum growth with approximately 4 γ of β-alanine per tube, in the following medium: casein hydrolysate 10 gm., ethanol 7 ml., lactic acid 15 gm., tryptophane 200 mg., nicotinic acid 2 mg., pimelic acid 150 γ; and $MgSO_4 \cdot 7H_2O$, KCl, $CaCO_3$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnCl_2$ and $MnCl_2 \cdot 4H_2O$ 1000, 400, 250, 5, 5, 5, and 2.5 mg., respectively, per liter, at pH 7.5. The amino acids in the medium do not interfere with the response to β-alanine. Growth ranges from an optical density of about 0.08 in the blank tubes to about 0.5 in the presence of 15 γ β-alanine, after 14 hours incubation at 37°C. Although pantothenic acid is five to ten times as active on a weight basis, the free vitamin is quantitatively destroyed by mild acid or basic hydrolysis. The total β-alanine is determined after appropriate treatment of samples.

Simultaneous biliary and urinary exogenous estrogen excretion studies in man. BENJAMIN F. STIMMEL. *Rees-Stealy Clinic Research Fndn., San Diego, Calif.*

Our modified procedure (*Federation Proc.* 9: 235, 1950) for the fractionation and photometric estimation of urinary estrogens has been adapted to human bile. The bile is mixed with Celite filter-aid (5% by weight) and an antifoam (Foamrex—4% by volume) and desiccated under reduced pressure. The fine powder is extracted successively with petroleum ether (extract discarded), 100% acetone (extract contains unconjugated estrogens), and 7-3 acetone-water mixture (extract contains conjugated estrogens). The phenol-like fractions from these acetone extracts are prepared for chromatographic fractionation and spectrophotometric estimation of estrone, estradiol, and estriol. Satisfactory recovery of the estrogens from bile to which sodium estrone sulfate (synthetic) and sodium estriol monoglucuronide were added, was achieved. Patients with drainage tube inserted in the common bile duct following cholecystectomy were treated with 30 mg. of the natural estrogens and the 48-hour post-treatment bile drainage (average volume 500 ml. per 48 hours, representing one-half to one-third of the normal hepatic output) and urine specimen were separately analyzed spectrophotometrically for estrone, estradiol, and estriol. In one case, 3% of the ingested estriol was recovered in the bile specimen and 11.5% in the urine. Estrone and estradiol could not be demonstrated in either fluid. In another similar study 7.7% and 27% of the ingested estriol were recovered in the bile and urine, respectively. Both free and conjugated forms of estriol were present in the bile (ratio of free to conjugated, was 1/2). A similar dose (30 mg.) of sodium estrone sulfate (synthetic) yielded only traces of estrone (50 μg.) and estriol (54 μg.) in the bile whereas the urine contained 204, 52, and 470 μg., respectively of estrone, estradiol, and estriol.

Effect of zinc hydrochloric acid hydrolysis on formaldehydogenic steroids in human urine. BENJAMIN F. STIMMEL. *Rees-Stealy Clinic Research Fndn., San Diego, Calif.*

In our procedure for the fractionation and photometric estimation of urinary estrogens (*J. Biol. Chem.* 162: 99, 1946), a neutral steroid fraction is

obtained as a by-product. Following zinc-hydrochloric hydrolysis of human urine this neutral steroid fraction, in spite of the comparatively drastic conditions of temperature (100 C for 3 hours), acidity (15 vol. % concentrated hydrochloric acid), and aqueous alkali (1 N NaOH) to which it is subjected, consistently yielded higher formaldehydogenic titers than were obtained from an aliquot of urine which was acidified to pH 1.0 and processed according to the procedure of Corcoran and Page (*J. Lab. & Clin. Med.* 33: 1326, 1948). Furthermore, more than 90% of the formaldehydogenic capacity appeared in the non-ketonic fraction following treatment of the neutral steroid residue with Girard's reagent T. To obtain further information on the effect of zinc-hydrochloric acid hydrolysis on urinary formaldehydogenic steroids, aliquots of pooled men's urine were refluxed for 10, 20, 30, 60, 120, and 180 minutes following the addition of 15 vol. % concentrated hydrochloric acid and 4 gm. % zinc dust, and then processed according to the procedure of Corcoran and Page. Maximal formaldehydogenic titers were obtained after 20 minutes hydrolysis and remained practically uniform up to 180 minutes. The maximal titer by zinc-hydrochloric acid hydrolysis were four- to fivefold that obtained on an aliquot of the urine acidified to pH 1.0, and similarly processed.

Hemin and globin synthesis in beryllium-induced anemia studied with alpha-C^{14}-acetate. H. E. Stokinger, K. I. Altman and K. Salomon (introduced by Alexander L. Dounce). *Dept. of Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*

Previous findings in anemias resulting from acute starvation or exposure to x-radiation have shown interference with hemoglobin synthesis. Since, in the aforementioned anemias, hemin synthesis was more markedly affected than globin synthesis, it was thought of interest to investigate other types of anemias in order to establish on a broader basis the causal relationship between anemia and biosynthetic activity. Another type of anemia investigated was that associated with acute, experimental beryllium poisoning. This anemia resembles a macrocytic type, is mild in degree, and is one of the most characteristic and consistent findings resulting from repeated, daily, 6-hour inhalation of beryllium dusts or mists. Three beryllium-exposed albino rabbits, which had a reduction of from $1 - 1.4 \times 10^6$ red cells/cμ. mm. and a mean corpuscular volume of from 86 — 100 cu.μ, were injected with 25 μc. alpha-C^{14}-acetate per kg. body weight. Blood samples were withdrawn at suitable intervals thereafter and hemin (as protoporphyrindimethylester) and globin were isolated for C^{14} analysis. A comparison with three hematologically normal control rabbits revealed that the rate of hemin and globin synthesis is significantly slower in all three beryllium-exposed rabbits. The available data indicate that the rate of globin synthesis is more markedly affected than the rate of hemin synthesis. (This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.)

Light production by green plants. Bernard Strehler and William Arnold (introduced by Waldo E. Cohn). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

It has now been demonstrated that green plants emit light for some time after they are illuminated. It is believed that this light may represent the reversal of early reaction in photosynthesis and is chemiluminescence rather than phosphorescence. The color is similar to, if not identical with, the fluorescence of the intact plant and the light has been detected for about 2 minutes following illumination. The intensity of the emitted light is about 10^{-3} of the fluorescent light below saturation. The decay curve at 6°C. follows reaction order of 2.0 while at 28°C. it follows reaction order 1.6. Ability to luminesce is irreversibly destroyed by temperatures of 50°C. while the temperature dependence is characteristic of an enzymatic reaction with an optimum at 37°C. and has an activation energy of 19,500 cal. The light energy may be stored even at low temperatures while higher temperatures are necessary for light emission. This light saturates, as does photosynthesis at higher light intensities, and the luminescence action spectrum closely follows the photosynthesis action spectrum. Carbon dioxide depresses the luminescence about 30% and continuous illumination shows an initial peak as does the fluorescence induction period. Of the inhibitors tested, cyanide, azide, thymol, and alcohol showed stimulation at low concentrations and inhibition at high concentrations while dinitrophenol, 2-methyl-1,4-naphthoquinone, and hydroxylamine·HCl were inhibitory at all concentrations. Ultraviolet light (2357 A) destroys ability to luminescence at about the same rate as it destroys photosynthesis.

Catalytic oxidation of chemically reduced diphosphopyridine nucleotide by cupric ion and cyanide. Jack L. Strominger (introduced by Robert F. Furchgott). *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

It was noted that the addition of 10^{-3} M cyanide to 10^{-4} M DPNH (reduced with $Na_2S_2O_4$) in 1 M phosphate buffer resulted in an exceedingly rapid oxidation of the DPNH, as measured by the disappearance of the 340 mμ absorption, thus confirming Slater (*Biochem. J.* 46: 484, 1950). A given

amount of cyanide could catalyze the oxidation of many times its equivalence of DPNH. There was a sharp optimum at about pH 7. In dilute phosphate buffer (0.01 M) the effect was much less than in 1 M buffer. In dilute buffer the addition of 10^{-6} M $CuSO_4$ produced a slow oxidation of DPNH, and the addition of 10^{-3} M cyanide to the $CuSO_4$ increased the rate at least 40-fold, bringing the rate to the maximum observed in 1 M phosphate buffer on addition of cyanide. With 4×10^{-5} M $CuSO_4$ plus cyanide the reaction was complete in less than a minute. Both this oxidation and that in strong phosphate are oxygen dependent and inhibited by 10^{-4} M cysteine. With ferric salts or ferricyanide at 10^{-4} M the oxidation was comparatively slow. Cyanate and thiocyanate cannot substitute for cyanide. In initial experiments, DPNH prepared with crystalline phosphoglyceraldehyde dehydrogenase could not be oxidized in this manner due to the presence of cysteine. Some of the reputed differences between DPNH prepared chemically and enzymatically may be due to the presence of compounds in the latter preparations protecting against heavy metal oxidations. (All concentrations refer to final concentrations.)
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Transaminases in higher plants. P. K. Stumpf. *Div. of Plant Biochemistry, Univ. of California, Berkeley.*

By employing ascending paper chromatography as the analytical tool, it has been observed that dialyzed aqueous extracts of lima bean, lupine, pumpkin and pea seedlings as well as pumpkin leaf extracts and unheated wheat germ are able to catalyze the transfer of amino groups of alanine, aspartic acid, α-aminobutyric acid, leucine, isoleucine, valine and norvaline to α-ketoglutaric acid to form glutamic acid. Boiled enzyme systems and systems complete except for the absence of either α-ketoglutaric acid or the specific amino acid failed to show transaminase activity. Oxaloacetic acid and pyruvic acid were inactive as amino acceptors. Tyrosine, phenylalanine, glycine, threonine, methionine, arginine, tryptophane and histidine had little if any activity as amino donors to α-ketoglutaric acid. Of some interest is the consistent observation that in all extracts *d*-alanine (free of *l*-alanine as determined by theoretical oxygen uptake studies with *d*-amino acid oxidase) behaves as an active amino donor. Further work will decide whether its activity is of a direct or indirect nature. Since at least seven amino acids may be oxidized to their corresponding keto acids by being coupled to the α-ketoglutaric-glutamic dehydrogenase system, no *l*-amino acid oxidase need be postulated for these amino acids in higher plants.

Purification of liver phosphorylase. Earl W. Sutherland. *Dept. of Biochemistry, Washington Univ. School of Medicine, St. Louis, Mo.*

Liver contains an enzyme which destroys phosphorylase rapidly. This enzyme is inhibited by 0.1 M fluoride or by muscle adenylic acid in low concentration; yeast adenylic acid is ineffective. Muscle adenylic, but not yeast adenylic acid, protects liver phosphorylase from heat denaturation. Use was made of these observations in the preparative procedure outlined below. Acetone powder of dog liver was extracted with 9 volumes of cold 0.1 M fluoride, the pH adjusted to 5.6 and the precipitate discarded. The fraction precipitated from the neutralized supernatant fluid between 0.33 and 0.6 saturation with ammonium sulfate was collected, dissolved in 0.1 M fluoride, and heated at 55° for 5 minutes in the presence of 1×10^{-3} M adenylic acid. The heat treatment inactivates the destructive enzyme. Further fractionation with ammonium sulfate and with alcohol at low temperature yielded a phosphorylase preparation which was (per mg. protein) about 200 times as active as the original liver homogenate. The purified liver phosphorylase formed from glucose-1-phosphate a polysaccharide which gave a pure blue color with iodine. This indicates that liver phosphorylase (like muscle phosphorylase) is specific for the formation of 1–4 linkages. The branching enzyme of liver (*J. Biol. Chem.* 151: 57, 1943), which is necessary for the formation of 1–6 linkages in glycogen, is separated from phosphorylase in this fractionation procedure.

Substrate specificity of pancreatic cholesterol esterase. Leon Swell,* Joan W. Cassidy* and C. R. Treadwell. *Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington, D. C.*

The hydrolytic and synthetic activity of pancreatic cholesterol esterase in respect to a series of 14 fatty acid esters of cholesterol were determined with methods described previously (*J. Biol. Chem.* 180: 615, 1949; 182: 479, 1950). The optimum pH for hydrolysis was the same for all members of the series; namely 6.55. The hydrolytic activity was greatest with esters of the fatty acids containing from 2–8 carbons. The highest rate of hydrolysis was observed with cholesterol caproate. The esters of fatty acids with more than 8 carbons were hydrolyzed at proportionately lower rates as the length of the carbon chain increased. Branching of the fatty acid chain markedly decreased the rate from that observed with the corresponding *n*-acid. For esterification, the optimum pH was in the range of pH 5.0 to 5.2 for all members of the series except stearic and oleic acids, which were 5.5 and 5.1, respectively. In further experiments it was found that the optimum pH for esterification

as determined for the fatty acids from acetic to palmitic acids probably did not represent the true optimum pH inasmuch as the enzyme was partially inactivated during incubation at acidities below pH 5.0. The results suggest that the true optimum pH for esterification varies with the fatty acid component. Summation experiments were carried out using a long- and a short-chain fatty acid ester and a mixture of the two esters. The total hydrolysis observed with the mixture of esters was less than the hydrolysis observed with the short-chain acid ester alone. Thus, the findings do not support the hypothesis that the short- and long-chain acid esters are hydrolyzed by different enzymes, but rather they suggest that the long-chain acid esters inhibit the hydrolysis of the short-chain acid esters.

Effect of ethionine on the choline oxidase system. MARIAN E. SWENDSEID, ANN LOUISE SWANSON* AND FRANK H. BETHELL.* *Simpson Memorial Inst., Univ. of Michigan, Ann Arbor.*

The effect of ethionine on a specific enzyme system, choline oxidase, has been studied using rat liver homogenates as an enzyme source. The rate of oxidation of choline was determined when ethionine was either added *in vitro* or fed in *in vivo* experiments. It was found that in both instances, ethionine inhibited choline oxidation. In *in vitro* experiments using 1:25 liver homogenates, the rate of choline oxidation as measured in the Warburg could be reduced by one-half when ethionine was added in equimolar concentration with choline. This inhibition was not reversed by methionine under the conditions employed but could be prevented by previous dialysis of the liver homogenate. In *in vivo* experiments, rats were fed an ethionine-containing diet for a 4-week period. When the choline oxidase content of liver homogenates from these rats was quantitated in the Warburg, values were approximately 10% of those which were obtained from livers of rats fed a similar diet without ethionine. The rate of choline oxidation in these livers could not be restored by dialysis of the homogenate.

Microbiological formation of vitamin B_{12} in fishery waste materials. H. L. A. TARR. *Pacific Fisheries Exptl. Station, Vancouver, B. C.*

Since vitamin B_{12} has been produced microbiologically largely by commercial concerns little information has been made available regarding substrates employed or yields obtained. Though this vitamin occurs abundantly in most fish tissues, especially in the viscera, its distribution in these and in by-products such as fish meals and condensed solubles is very variable (TARR *et al. Food Technology* 4: 354, 1950). With a view to increasing, and possibly standardizing, the vitamin B_{12} content of such products *Streptomyces griseus* and *S. aureofaciens* have been cultured with simple aeration in herring press water adjusted to 2% solids content and with no additive other than 2 μg/gm. of cobalt. Microbiological determination of vitamin B_{12} in the crude fermentation liquids indicated that maximum yields were obtained after about 4 days at 27°. No agreement whatever was obtained in the results of assays of the crude fermentation products with *Lactobacillus leichmannii* in tube turbidimetric procedure and with a cup-plate assay employing a glucose-inorganic salt medium inoculated with a vitamin B_{12} requiring mutant of *Eschrichia coli*. In general the vitamin B_{12} content as indicated by *L. leichmannii* assay (about 400 mμg/ml) was somewhat similar to that obtained following chromatographic separation on KH_2PO_4 impregnated filter paper, development on agar inoculated with the *E. coli* mutant, and subsequent assay of the eluted vitamin. It appears that the hydroxy cobolamine form of vitamin B_{12} occurs generally in these fermentation products. On treatment with KCN (2.5 mg/ml.) at pH 5.5 about one-half the total activity of the crude products appears as vitamin B_{12} following chromatographic separation. All activity disappears on treatment with 0.2 N NaOH at 100° for 30 minutes.

Metabolism of plasma protein. HAROLD TARVER AND ISMAIL A. ABDOU. *Div. of Biochemistry, Univ. of California, Berkeley.*

The metabolism of plasma protein has been followed in rats by administering plasma labeled by giving donor animals serine-β-C^{14}. Plasma taken from the donors after 8 hours and subjected to dialysis had label in the protein fraction exclusively. After administration by vein (i.v.) to 200-gm. recipients in 1.5 ml. doses the concentration of the label in the plasma decreased at rates corresponding to apparent half-times of 1.6, 11 and 74 hours. The slowest rate is attributed to plasma protein renewal. Six hours after giving the same dose intraperitoneally (i.p.) the label concentration was the same in the plasma protein as that after i.v. injection. Doses given orally never led to comparably high labeling. The rate of labeled carbon dioxide production reached about the same maximum value almost at the identical time whether the protein was given i.v. or i.p., but the rate after oral administration was much higher in the early hours and lower later (after 12 hours). Little of the label from plasma was lost via other channels of excretion. Animals subjected to *in vivo* perfusion immediately after the injection of the protein, had considerable residual activity in tissues, especially in the liver, kidney and spleen. Activities in the tissues of animals sacrificed at intervals up to 168 hours have been measured. The

route of administration proved to have a smaller effect on the distribution of the label than might have been anticipated had plasma protein been in direct equilibrium with all tissues and their component proteins.

Spirocheticidal substances of syphilitic blood. HENRY TAUBER, CHARLOTTE MCLEOD,* WARFIELD GARSON* AND HAROLD J. MAGNUSON. *Venereal Disease Exptl. Lab., U. S. Public Health Service, Univ. of North Carolina School of Public Health, Chapel Hill.*

Spirocheticidal substances, presumably antibodies, in the serum of patients and animals with syphilis has been demonstrated by a succession of workers in the past 30 years. In the early investigations, the spirocheticidal effect was demonstrated *in vivo;* more recently, however, Nelson and Mayer have developed *in vitro* methods for the measurement of this effect. In the present study an attempt is made to isolate and identify the spirocheticidal substances. Cohn's Method 10 of plasma fractionation has been adapted to the isolation of the various components of syphilitic plasma and serums. Normal blood samples (plasma and serum) were employed as controls. The chemical fractions were analysed by electrophoresis. Fractionations have been performed on specimens from approximately 50 patients with untreated early syphilis in whom Treponemal antibodies could be demonstrated. The spirocheticidal substances were present in Precipitate I + III-1, 2, 3 associated with the high molecular weight isoagglutinins and a small amount of prothrombin protein (and fibrinogen when plasma was employed); and in Precipitate I + III-3 associated with the high molecular weight antihemophilic globulin and cold insoluble globulin and a small amount of plasminogen. Both precipitates represent a small percentage of the blood proteins. These precipitates apparently contain nearly all of the spirocheticidal substances.

Metabolism of C^{14}-labeled glycerol and C^{14}-labeled pyruvate by rat liver, in vitro. CHING-TSENG TENG,* A. BAIRD HASTINGS AND FRANCES B. NESBETT. *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Experiments on the metabolism of α-carbon labeled glycerol and α-carbon labeled pyruvate have been carried out on normal rat liver slices *in vitro* in a potassium-rich medium containing both glycerol and pyruvate as substrates. From the data obtained, the amount of glycerol and of pyruvate carbons which converted to glycogen, glucose, lactate, CO_2, barium and alcohol precipitable fractions, lipids, and protein residue have been calculated. These account for 92% of the glycerol carbons and 57% of the pyruvate carbons which are metabolized. About three times as much glycerol is converted to glucose and glycogen as is pyruvate; on the other hand, about three times as much pyruvate is converted to lactate and CO_2 as is glycerol. A comparison has also been made between the metabolism of pyruvate carbons in glycerol-pyruvate media and in glucose-pyruvate media. In the latter media, about 62 per cent of the pyruvate carbons metabolized are accounted for. In the glucose-pyruvate media, more of the pyruvate carbons were converted to glycogen and CO_2; less were converted to lactate; and about the same number were converted to glucose. These results provide quantitative data on the extent to which glycerol may participate in the formation of glucose, glycogen and CO_2, and in addition, the extent to which glycerol and pyruvate undergo dismutation *in vitro*.

Determination of coenzyme A. L. J. TEPLY AND WINIFRED ROGERS.* *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

V. Jagganathan, J. C. Rabinowitz and R. S. Schweet have observed that in the presence of a purified pyruvic acid oxidase system, pigeon liver preparations with coenzyme A added acetylate sulfanilamide. It is not necessary to add ATP. This procedure has been used with the 4-aminoazobenzene acetylation assay. Under these conditions, synthetic panthetine (LBF) (obtained from Dr. E. E. Snell) has been found to be completely inactive, though it does show some activity if ATP is added. The assay has been applied to fractions obtained during coenzyme A purification. The use of arsenate to distinguish between different pantothenic acid compounds in the acetylation of 4-aminoazobenzene has not been satisfactory in our hands.

Effect of choline on fat absorption. H. C. TIDWELL AND MARY E. NAGLER.* *Biochemistry Dept., Southwestern Med. School, Dallas, Tex.*

Choline has been shown to aid the passage of fat from the intestine to the lacteals and to increase the rate of the appearance of fat in the blood. The question was raised as to whether this action might be the result of an acetylcholine-like effect of the choline rather than that of promoting phospholipid formation. Hence the effect on the rate of fat absorption of varying amounts of prostigmine and mecholyl, which might produce a similar physiological effect, was studied. Very small amounts of mecholyl appeared to exert an effect similar to that of choline, while larger amounts of both mecholyl and prostigmine retarded the appearance of the absorbed fat in the blood. If supplements of methionine or methionine and ethanolamine should hasten the rate of fat absorption, it might be that they were promoting

choline and possibly phospholipid formation. It would be supporting evidence against only an acetylcholine-like action with choline. Preliminary results do not indicate that these supplements enhance the rate of fat absorption. However, choline formation may be too slow to show an effect during a 3-hour period. Studies to date would suggest that at least a part of the effect of choline on fat absorption was due to its acetylcholine-like action.

Light-scattering studies on insulin. FRANK TIETZE* AND HANS NEURATH. *Dept. of Biochemistry, Univ. of Washington School of Medicine, Seattle.*

In a recent investigation of the disaggregation of insulin in acid solution by the methods of ultracentrifugation and diffusion, Fredericq and Neurath have assigned a minimum molecular weight of 6000 to this protein. Since this value is in disagreement with those previously reported by others (about 12,000), the protein has been subjected to further investigation by the method of light-scattering. In a preliminary investigation with proteins of known molecular weight, satisfactory values were obtained with crystalline bovine serum albumin (70,000 ± 3000) and chymotrypsinogen (26,000 ± 1000). In phosphate buffer of pH 2.6 and ionic strengths 0.1, 0.05, and 0.01, and in NaCl solution of the same pH and ionic strength 0.1, extrapolation of the scattering data to infinite dilution yielded molecular weights of 12,000 ± 1000. Analysis of the scattering curves leads to the following conclusions: *1*) at any particular insulin concentration the extent of aggregegation with increasing ionic strength of phosphate buffer; *2*) aggregation of insulin is more pronounced in NaCl solution of ionic strength 0.1 than in phosphate solution of the same ionic strength. While these results confirm those of Ellenbogen and Oncley and of Gutfreund, they cannot be considered conclusive because of the well-known sensitivity of the light-scattering method to small amounts of impurity of high molecular weight. Thus only 5% of an impurity of molecular weight 32,800 is sufficient to raise the extrapolated molecular weight from 6000 to 12,000 in the light-scattering method.

Comparative rates of renewal of choline and phosphate groups in the phospholipids of rat liver. MARIAN TOLBERT* AND RUTH OKEY. *Dept. of Home Economics, Univ. of California, Berkeley.*

Intact 200-gm. male rats were injected simultaneously with tracer doses of C^{14} methyl labeled choline (supplied by Drs. M. Calvin and B. M. Tolbert of the Radiation laboratory) and P^{32} labeled phosphate. At the desired time intervals thereafter, the rats were sacrificed and the quantity and activity of the choline and phosphate in the various liver fractions determined. Choline was separated as the reineckate and phosphorus as $MgNH_4PO_4$ before counting. Calculation of turnover time of the choline containing phospholipid phosphate from specific activity-time relationships of precursor and compound for 19 rats (by the method of ZILVERSMIT, D. D., C. ENTENMAN AND M. C. FISHLER. *J. Gen. Physiol.* 26: 325, 1943) using glycerophosphate as a precursor, gave a mean value of 10.9 hours. Non-phospholipid choline proved not to meet the criteria for a precursor of phospholipid choline. Comparison of rate of renewal on the basis of decay as estimated by plotting the best fitting straight lines for logarithmic specific activity values was therefore attempted. Data from 20 additional rats were used. After 10 hours the slopes for phospholipid phosphate and for phospholipid choline were similar, as were those for inorganic phosphate and non-phospholipid choline. There was, however, some indication that the labeled phospholipid choline had an earlier and a slightly higher maximum concentration than did the labeled phospholipid phosphate.

Influence of triphosphopyridine nucleotide upon evolution of oxygen and fixation of carbon dioxide by illuminated chloroplasts. L. J. TOLMACH* AND H. GAFFRON. *Dept. of Chemistry and Inst. of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill.*

Illuminated spinach juice has been shown to evolve small amounts of oxygen. In a study of this phenomenon, it was found that the reaction requires substances in the cell sap in addition to the photochemical apparatus of the chloroplasts. Oxygen evolution from juice was increased by the addition of the photosynthetic intermediate phosphoglyceric acid, as well as by addition of pyruvic acid and hexose diphosphate, with oxygen appearing in yields up to 16% of the amount calculated on the basis of added substrate. Addition of TPN gave much greater stimulation, with yields over 30 times the stoichiometric. None of these substances appeared to be effective in the presence of separated chloroplasts. The reduction of TPN by chloroplasts in a light reaction was demonstrated with the aid of the 'malic' enzyme system. Under these conditions oxygen evolution appears coupled with a fixation of carbon dioxide, as shown by radioactive tracer experiments. The reduction potential of TPN is several tenths of a volt below those of the ordinary Hill reagents; its reduction provides a method for the coupling of CO_2 reduction with the photochemical activity of chloroplasts.

Alkali-catalyzed transformation of glucose into fructose and mannose. YALE J. TOPPER (introduced by DEWITT STETTEN, JR.). *Div. of Nutrition and Physiology, Public Health Research Inst. of the City of New York, Inc.*

The alkali-catalyzed transformation of glucose into fructose and mannose has been studied with the aid of deuterium. The distribution of isotope in the products has been ascertained when the source of isotope was either D_2O or 1-deuterio-glucose. In the former case, it has been demonstrated that the fructose which was formed in saturated lime-water had exchanged 1.68 atoms of hydrogen at 35° and 1.55 atoms at 25°. Thus, in contrast to those of others, the present findings are in complete accord with the concept that the transformation proceeds via enolization, both at 25° and at 35°. When 1-deuterio-glucose was used as the source of isotope it was found that, in successive experiments, the mannose formed had retained 46, 44 and 52% of the isotope contained in the starting material, while the fructose retained 94 and 100%. These results indicate that the mannose which arose when glucose was treated with a limited amount of alkali (i.e. saturated lime-water) is derived exclusively from fructose. From this it is concluded that the transformation proceeds over two distinct steps: glucose ⇄ *trans*-enediol ⇄ fructose, and fructose ⇄ *cis*-enediol ⇄ mannose. The provisional assignments of *trans*- and *cis*-enediols is based on the relative acidic strengths of the three hexoses. The possible relationship of these findings to the enzymic isomerizations of the phosphohexoses will be discussed.

Influence of folic acid on metabolism of glycine, serine and threonine by *Streptococcus faecalis*. JOHN R. TOTTER AND DOROTHY S. GAINES.* *Univ. of Arkansas School of Medicine, Little Rock.*

Investigation of the behavior of *Streptococcus faecalis* toward high concentrations of amino acids has revealed that inhibition of growth by glycine and threonine is much more pronounced when the folic acid concentration is also elevated. With 0.5 mμg. of folic acid per ml. of medium, glycine in 35 mM and DL-threonine at about 100 mM concentration reduced the maximum growth by one-half. On the other hand when folic acid was present at 20 mμg. per ml. of medium 5–7 mM glycine and 13 mM DL-threonine brought about a 50% reduction in maximum growth. Three and five-tenths mM DL-serine produced a similar inhibition which, however was independent of the folic acid concentration. DL-alanine, DL-phenylalanine, L-lysine, L-arginine, DL-aspartic acid, DL-norleucine, DL-leucine do not inhibit growth of the organism under similar conditions. Histidine and glutamic acid inhibited growth but the mechanism appears to be different. The inhibitory effect of high folic acid concentration can be partially reversed by aminopterin. The glycine effect was reversed by dimethylglycine which also appears to be able to replace folic acid for growth of *S. faecalis*. The breakdown of glycine appears to be limited by the available folic acid. The inhibitory substance produced from glycine in the presence of large quantities of folic acid may be serine. The results also suggest that threonine is converted to glycine by *S. faecalis*.

Anomaly in acetylcholine system in cerebral cortex in focal epilepsy and following methionine sulfoximine administration. D. B. TOWER* AND K. A. C. ELLIOTT. *Montreal Neurological Inst., Montreal, Quebec, Canada.*

Determinations have been made, on cerebral cortex from various unanesthetized mammals, of total acetylcholine (ACh) content, cholinesterase activity, and the rates of increase of bound and of free ACh with slices incubated in the presence and absence of a high potassium concentration. Per unit weight, all these quantities decrease regularly and to the same relative extent with increasing brain weight of the animal. In human focal epileptogenic tissue there is a definite failure of the bound ACh to increase on incubation and a probably significant small increase in cholinesterase activity. Other aspects of the ACh system fall in the probable normal range. A similar failure of bound ACh production occurs in tissue from animals in which a convulsive condition has been produced by administration of methionine sulfoximine. (This substance, 'agene toxic factor,' was kindly supplied by Dr. L. Reiner, Wallace and Tiernan Products, Inc.) Partial anoxia *in vitro* prevents the increase in bound ACh in incubated slices of normal cortex without affecting the production of free ACh. Glutamine or methionine added to the suspending medium reverses the effect of methionine sulfoximine administration. Glutamine, but not methionine, reverses the effect of partial anoxia. Administration or pentobarbital, ether, tribromoethanol, Dilantin or phenobarbital causes an increase in bound ACh production and counteracts the effect of partial anoxia.

Color reactions of amino and imino acids with ninhydrin. WALTER TROLL* AND R. KEITH CANNAN. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

All published colorimetric procedures involving ninhydrin suffer from variable non-quantitative recovery of color from different amino acids. An attempt has been made to overcome this. We find that the rate of the reaction is increased in organic solvents containing less than 10% water and particularly in mixtures of phenol and pyridine. In a

system containing ninhydrin, alcohol, pyridine, phenol, potassium cyanide and water almost all amino acids as well as small peptides are converted quantitatively to diketohydrindylidene-hydrindamine in 1–3 minutes at 100°. Glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, phenylalanine, tyrosine, glutamic acid, aspartic acid, arginine and histidine give 97–102% yield of the colored derivative as measured in the Beckmann spectrophotometer. Lysine gives a somewhat high value per mole, tryptophane a low value. The reagents are supplied from three stable stock solutions. The imino acids, proline and hydroxyproline, react differently. The first product is a red compound which appears to be identical with the compound isolated by Grassman and Arnim (*Ann. Chem.* 509: 288, 1934). The red derivative of proline is very rapidly converted to a yellow product whereas that of hydroxyproline reacts only slowly. A specific method of analysis of hydroxyproline has been based on this difference in behavior.

Standards for protein-bound iodine concentration in the serum of men of certain age groups. ROBERT G. TUCKER (introduced by ANCEL KEYS). *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

Serum protein-bound iodine (PTI) determinations were made in 404 urban Minnesota men who were found clinically normal in detailed examinations. There were 130 university students (18–25 years of age) and 274 business and professional men (45–56 years of age). The frequency distribution curve for PBI was significantly skewed and leptokurtic so percentile values instead of standard deviations were used to define the limits for possible and probable abnormalities. No correlation was observed between age and the serum PBI within the young group or within the middle-aged group. However, the young group of normal men was significantly higher in serum PBI than the middle-aged group. A low but significant correlation was demonstrated between the serum PBI and the BMR ($r = +0.112$). A low but significant linear correlation was observed between the serum PBI and the serum total cholesterol concentration in the middle aged group of normal men ($r = +0.158$); no such relation was found in the group of normal young men. For the gross sample of normal men a curvilinear relationship was demonstrated between the serum PBI and the serum cholesterol, presumably reflecting the relationship of both factors to age. No relationship was found between the serum PBI and the relative obesity expressed as the deviation of actual body weight from the 'normal' in standard tables.

Purification and amino acid content of pressor principle (vasopressin) of posterior lobe of the pituitary. ROBERT A. TURNER,* JOHN G. PIERCE* AND VINCENT DU VIGNEAUD. *Dept. of Biochemistry, Cornell Univ. Med. College, New York City.*

A method has been developed for obtaining highly potent vasopressin preparations. This method consists of counter-current distribution in an all-glass apparatus (CRAIG AND POST. *Anal. Chem.* 21: 500, 1949) with a solvent system of *n*-butyl alcohol and aqueous *p*-toluenesulfonic acid. After distribution, the acid is removed from the active fractions with Amberlite IR-4B resin. Preparations of high potency, 400–500 pressor U/mg., can be obtained by these procedures. Analysis of these preparations by starch column chromatography shows the presence of phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, ammonia, arginine and cystine. Moreover the eight amino acids are in molar ratios to each other of approximately 1:1 with about 3 moles of ammohia for 1 mole of each of the amino acids. Only slight traces of other amino acids were found. In terms of these 8 amino acid residues and ammonia, 91% of the weight of unhydrolyzed compound was accounted for. Whether the portion unaccounted for is due to the presence of impurities not detected by the analytical procedure or due to other moieties in vasopressin not detected remains for future investigation.

Renal excretion of administered radiophosphorus following thyroparathyroidectomy and the injection of parathyroid extract. W. R. TWEEDY AND E. M. ZORN.* *Radioisotope Unit, Veterans Administration Hosp., Hines, Ill.*

During the 3 days immediately preceding thyroparathyroidectomy, restriction of the daily food (Purina laboratory chow) intake of 130 rats, weighing 100–200 gm., to approximately one-half the amount they normally would have consumed, accentuated the retention of the P^{32} that was administered after the operation. The subcutaneous injection of a single dose of parathyroid extract (Lilly) (0.5, 1.0, 2.5, or 5 U. S. P. U), 18 hours after the 65 experimental animals had been operated on and 20 minutes before the intraperitoneal injection of 15 μc. of essentially carrier-free P^{32} in both the control and the experimental animals was followed, during the next 5 hours, by the urinary excretion of 11–15% (statistically significant mean values) of the dose of P^{32} as compared to 3–6% excreted by the operated controls. Comparison of the serum calcium values of the experimental animals that received 0.5 U of parathyroid extract and their controls at the end of the 5-hour period revealed no increase in the serum calcium. Injection of small amounts of parathyroid extract,

added to normal rat or human blood serum, produced the expected increase in the urinary excretion of P^{32}, but normal blood serum alone or blood serum suspected of containing an increased amount of parathyroid hormone activity was ineffective.

Rates of synthesis and turnover of proteins and nucleic acid purines in the rat. EVELYN P. TYNER,* CHARLES HEIDELBERGER AND G. A. LEPAGE. *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

The studies of LePage and Heidelberger (*Federation Proc.* 9: 195, 1950) have been extended by an investigation of the rate of incorporation of glycine-2-C^{14} into the nucleic acids and proteins of the rat, and the subsequent turnover rate of the purine moieties. Labeled glycine was administered by stomach tube to rats bearing multiple 10-day Flexner-Jobling carcinoma transplants. After various intervals of time the animals were sacrificed. The protein, desoxyribonucleic acid (DNA), and pentose nucleic acid (PNA) were isolated from liver, kidney, and tumor tissue, and their specific radioactivities were determined. Adenine and guanine were isolated by chromatography after hydrolysis of the nucleic acids, and their specific activities as well as that of the free glycine in the tissues were determined. An appreciable proportion of the isotope was shown to be present in the DNA as well as in the PNA. The radioactivity was incorporated more rapidly into both protein and purines in liver than in tumor and least rapidly in kidney. These results are essentially in agreement with and supplement the work of Griffin *et al.* (*Cancer* 3: 316, 1950).

***Clostridium histolyticum* collagenase.** ALFRED A. TYTELL, JAMES D. OGLE AND KATHLEEN HEWSON (introduced by MILAN A. LOGAN). *Dept. of Biological Chemistry, College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio.*

A purified preparation has been made of a proteolytic enzyme which attacks native, fibrous collagen. This enzyme preparation is free from contamination with other proteinases and peptidase(s) present in original filtrates and in crude preparations. The activity of the purified collagenase is comparable to that of crystalline trypsin when gelatin is used as a substrate. However, collagenase liberates more free amino groups than crystalline trypsin for an equal drop in gelatin viscosity. Collagenase attacks α-benzoyl-L-argininamide. It does not attack denzoyl-glycinamide, benzoylglycyl-glycine, L-glutamyl-L-tyrosine, benzoyl-L-alaninamide, glycyl-L-leucine, L-leucinamide, L-leucylglycine, L-leucyl-diglycine, diglycine, glycinamide, triglycine, and L-prolinamide. There is an indication from micro-iontophoretic studies that collagenase and trypsin produce distinctly different peptide patterns in the digestion of gelatin. The kinetics of collagenase proteolytic activity on complex and synthetic substrates will be compared to those of the common mammalian crystalline proteolytic enzymes.

Enzymatic formation of tyrosine from phenylalanine. S. UDENFRIEND, J. COOPER* AND C. T. CLARK.* *Section on Chemical Pharmacology, Natl. Heart Inst., Bethesda, Md.*

Bernheim (*J. Biol. Chem.* 152: 481, 1944) observed the formation of a phenolic substance when rat liver slices were incubated with L-phenylalanine and suggested that the product could be tyrosine. Work in this laboratory has confirmed this finding for rats and demonstrated it in many other species as well. The identity of the phenolic substance with tyrosine has been confirmed by two procedures; *a*) treatment with tyrosine decarboxylase and identification of the resulting tyramine, and *b*) isolation of radioactive tyrosine chromatographically after C^{14} labelled phenylalanine had been incubated with liver slices. The system that carries out this transformation is found in the supernatant when liver homogenate is centrifuged at 15,000–20,000 *g* and requires D.P.N. or T.P.N. for activity. The crude enzyme preparation shows no activity with phenylglycine or phenylserine. Some activity is observed when o-phenylalanine is used as substrate but this may be due to recemization before reaction. Derivatives of L-phenylalanine with substitution on the nitrogen are also acted upon; acetyl-, chloracetyl-, and carbobenzoxyglycylphenylalanine are as active as the parent compound. Cyanide, azide, fluoride and arsenate have little effect on the activity but β-2-thienylalanine (.02M) strongly inhibits. The latter observation is of interest since this compound has been shown to act as an antimetabolite of phenylalanine in animals and microorganisms. Attempts are being made to purify the enzyme system and to investigate the mechanism of the reaction.

Investigation of Bence-Jones proteins. BERNARD UDIN* AND FRANK W. PUTNAM. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Physical chemical and immunological studies have been made of the urinary proteins from 3 patients with multiple myeloma all of whom exhibited a similar abnormal serum protein. Two excreted an abundant homogeneous Bence-Jones protein with physical constants for JM and RF respectively of: S_{20} = 3.60 and 3.44 Svedberg u; D_{20} = 7.7 and 7.6 $\times$ 10^{-5} cm.^{2} sec.$^{-1}$; f/fo = 1.16, and 1.19; molecular weight = 43,500, and 45,200; and mobility in veronal buffer pH 8.6 = 4.4 and 2.2 $\times$ 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}. S_{20} for the protein

from JM was independent of concentration over the 8-fold range studied. The molecular weight and f/fo of the Bence-Jones proteins differ significantly from literature values. Antiserum to the urinary protein from JM gave negligible cross reaction to that from RF. A urinary protein excreted in low amount by MS waselectrophoretically and ultracentrifugally heterogeneous, and did not give the typical Bence-Jones heat reaction. Ultracentrifugal study showed it consisted largely of low molecular weight substance with S_{20} of about 2.2. However, in all three cases the major abnormal serum component was similar electrophoretically and ultracentrifugally (mobility = 2.6–2.8 U at pH 8.6; S_{20} = 8.6–8.9S). This serum protein appears to be a discrete species, previously uncharacterized, and occurring occasionally in multiple myeloma. There is no correlation between the presence of this serum protein and the physical chemical properties of the urinary proteins. However, immunological relationships are being further investigated.

Action of crystalline trypsin on insulin and the hyperglycemic factor. F. R. VAN ABEELE AND E. D. CAMPBELL (introduced by OTTO K. BEHRENS). *Div. of Biochemistry, Eli Lilly Research Labs., Indianapolis, Ind.*

Although the hyperglycemic factor is completely destroyed by the action of crystalline trypsin, insulin is surprisingly resistant to this enzyme. It was possible to obtain insulin that was free of hyperglycemic activity by use of this reaction. Using trypsin to insulin ratios from 1:1000 to 1:15,000 by weight it was impossible to detect any loss in insulin potency after digestion. This reaction, although useful for the preparation of insulin which is devoid of hyperglycemic activity, has the disadvantage of causing the resulting insulin to be contaminated with trypsin. The resulting insulin-trypsin combination was very stable. A thorough reprocessing including crystallization failed to remove the enzyme from insulin. The unusual character of the mutual affinity of insulin and trypsin suggests an explanation of the resistance of insulin to the hydrolytic action of this enzyme. Various insulin preparations were assayed for proteolytic and hyperglycemic activity and it was found that insulins prepared by use of trypsin showed no hyperglycemic activity but appeared to have proteolytic activity, while insulin preparations made without the use of trypsin often contained hyperglycemic activity. At least some commercial insulin preparations which were known to contain no hyperglycemic factor appear to possess proteolytic activity.

Observations on solvation and reconstitution of collagen. PARKER VANAMEE AND KEITH R. PORTER (introduced by S. GRANICK). *Rockefeller Inst. for Med. Research, New York City.*

In an attempt to understand the structure and properties of collagen, stages in the solvation and reconstitution of rat tail tendon were examined with the electron microscope. Partial breakdown of the fibers was accomplished by enzymatic digestion with trypsin. This procedure revealed that the fibers were made up of bundles of filaments which were beaded in nature, groups of three beads giving the large period of collagen. Solvation of the collagen was also accomplished with dilute acetic acid and the fundamental fibrils thus obtained were reconstituted with varying concentrations of sodium chloride and hydrogen ion. At saline concentrations of 1% the fibers formed were tactoid in shape and showed both a small (210Å) and a large (640Å) periodicity, but never, precisely, the fine structure of natural collagen. Higher and lower concentrations of sodium chloride produced smaller fibrils showing little evidence of periodic structure. Rate of fiber formation varied directly with concentration of salt. Hydrogen ion concentration at the isoelectric point reported for collagen (pH 5.8) produced fibers having a fine structure indistinguishable from that of natural collagen. Hydrogen ion concentrations above and below this point yielded fibers showing significant variations from the normal in size and structure. Rate of fiber formation varied inversely with the concentration of hydrogen ion.

Metabolism of cholesterol: absorption, tissue distribution and fatty acid synthesis. J. T. VAN BRUGGEN,* T. T. HUTCHENS* AND E. S. WEST. *Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.*

C_{14} labeled, biosynthesized cholesterol has been fed via stomach tube to two young male rats. One received 316 mg. (57 counts/min/mg.), in 5 doses in oil. The other received 100 gm. (404 counts/min/mg.) in 4 doses in oil over 48 hours. Liver, skin, carcass, brain plus spinal cord, and gut and feces were separated into saponifiable and non-saponifiable fractions. Radioassay of the cholesterol fractions indicated all tissues contained C_{14} sterols in the descending order of total activity: gut, carcass, liver, skin and brain. Liver cholesterol had the highest specific activity (excluding gut cholesterol). Fatty acid fractions showed a C_{14} activity in the descending order for total activity: gut, skin, carcass, brain and liver. Brain fatty acids showed specific activities exceeded only by gut fatty acid fractions. Liver glycogen of the second experiment had a specific activity of 0.07 counts/min/mg. All activities are reported as for $BaCO_3$ at infinite thickness. Total tissue C_{14} was 12% of the administered cholesterol activity. The

activity of the fatty acids suggests that cholesterol is catabolized through a process that yields acetate fragments for fatty acid synthesis. At least 5% of the administered cholesterol activity was recovered in tissue fatty acids. The activity of the liver glycogen indicates that at least part of the molecule may enter carbohydrate synthesis cycles.

Determination of total and radioactive carbon. DONALD D. VAN SLYKE, ROBERT STEELE* AND JOHN PLAZIN.* *Depts. of Biology and Medicine, Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Total carbon is determined by the wet combustion method of Van Slyke and Folch, with manometric measurement of the CO_2, air-free reagents being used in the manometric chamber so that the zero manometric reading can be determined after ejecting the CO_2. The ejected CO_2 is used for measurement of C^{14} activity. For this two methods have been developed. In Method I the CO_2 is precipitated as $BaCO_3$. The $BaCO_3$ is filtered on a sintered glass disk and the activity counted in a methane flow counter. In Method II the CO_2 is transferred to the gas counter developed in this laboratory by Bernstein and Ballentine. Counting in the gas phase has the advantage that there is no self-absorption, the count being a linear function of the amount of $C^{14}O_2$ in the counter. With the $BaCO_3$ counter the useful range of total carbon sample size is 0.3–2.0 mg. Less than 0.3 mg. of carbon does not yield enough $BaCO_3$ for uniform layers, and because of self-absorption more than 2 mg. gives no greater count. With Method II, employing the gas counter, the useful range is 0.1–15.0 mg. of total carbon. With a given counting time and error, Method II permits measuring a specific carbon activity about 0.01 of that which can be measured by Method I. (Research sponsored by Atomic Energy Commission.)

Activity of glyceraldehyde-3-phosphate dehydrogenase and its binding of coenzyme and substrate. SIDNEY F. VELICK. *Dept. of Biochemistry, Washington Univ. School of Medicine, St. Louis, Mo.*

Glyceraldehyde dehydrogenase crystallizes from rabbit muscle with 1.7 molecules of DPN per molecule of protein (M.W. 120 000). When the DPN is removed with norite, the activity of the enzyme increases linearly with added DPN until 2 molecules of DPN per molecule of protein have been added and then remains constant. This indicates that 2 molecules of DPN are bound and that both binding sites are catalytically active. The linear velocity increment is a consequence of the great affinity of the enzyme for DPN. Binding of DPN was also measured by analysis of supernatant solutions after ultracentrifugation of enzyme-DPN mixtures. The sedimenting protein carries down 2 molecules of DPN, and a dissociation constant of the order of 10^{-10} M/ml. can be calculated. This apparent thermodynamic dissociation constant is about 100 times smaller than the Michaelis constant for DPN. The reasons for this difference will be discussed. Two molecules of reduced coenzyme (DPNH) are also bound by the protein. Direct quantitative observation of displacement of DPN by DPNH shows that the binding of DPN and DPNH is competitive and that the dissociation constants are the same. These conclusions are confirmed by kinetic analysis of the course of the reaction. The DPNH that is produced inhibits the reaction competitively to the extent expected if the dissociation constants of enzyme DPN and enzyme-DPNH were the same.

Action of vitamin B_{12} and folic acid on labile methyl synthesis in the rat. WALTER G. VERLY,* JOHN E. WILSON,* JOHN M. KINNEY* AND JULIAN R. RACHELE. *Dept. of Biochemistry, Cornell Univ. Med. College, New York City.*

It has been shown that the carbon of methanol can be incorporated into the methyl group of choline in the rat (DU VIGNEAUD AND VERLY. *J. Am. Chem. Soc.*, 72: 1049, 1950). Experiments with methanol labeled with deuterium and C^{14} indicated that methanol was used, presumably through oxidation and subsequent reduction, in the synthesis of a methyl group in the formation of a labile methyl compound (DU VIGNEAUD, VERLY, WILSON, RACHELE, RESSLER AND KINNEY. *J. Am. Chem. Soc.* In press). This newly formed methyl group then enters transmethylation reactions. Experiments have now been undertaken to see whether vitamin B_{12} or folic acid had any effect on the utilization of methanol. C^{14}-Deuteriomethanol was injected into rats subcutaneously. Trimethylamine derived from choline isolated from the carcasses was analyzed for C^{14} and deuterium. No significant difference in the utilization of methanol for labile methyl synthesis was found between young, B_{12}-deficient rats and their litter mates prepared in the same way but injected with B_{12} prior to administration of methanol. Folic acid administration caused a two-to-threefold increase in the utilization of methanol in labile methyl synthesis in the folic acid-deficient rat. These results on B_{12} and folic acid supplement those obtained by Sakami and Welch (*J. Biol. Chem.* 187: 379, 1950) on labile methyl synthesis from formate in liver slices.

Isolation of rat liver lactic dehydrogenase. CARL S. VESTLING, DAVID M. GIBSON,* EDWIN O. DAVISSON* AND B. ROGER RAY.* *Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.*

Studies of rat liver lactic dehydrogenase have shown it to be a very stable protein and thus well

suited for isolation work. A method for the separation of the enzyme in highly purified form has been developed. Fresh rat liver homogenates (28%) in 0.25 M NaCl-0.012 M $NaHCO_3$ have been made in a Waring blendor and then brought at 0° to 20% ethanol concentration, pH 6.5. Inert protein is removed, leaving a clear supernatant fluid. Subsequent purification is accomplished by operating at $(NH_4)_2SO_4$ saturation levels between 40 and 50% and at pH's in the range, 5.7–8.3. The enzyme remains soluble at $(NH_4)_2SO_4$ concentrations of 40% of saturation or less, while it is precipitated at saturation levels in excess of 50% in the pH range stated. Fractions have been obtained which show 200 times the specific activity of the original homogenates. Activity assays are conducted spectrophotometrically with enzyme, lactate, DPN and veronal buffer at pH 8.4. Ultracentrifugal analysis indicates the presence of two principal components in the purified material. Electrophoretic studies show the enzyme to be slow-moving with respect to contaminating proteins at pH 8.3. Further characterization of the purified fractions is in progress.

Metabolism of C^{14}-glucose and pyruvate-2-C^{14} by mammalian muscle in vitro. CLAUDE A. VILLEE AND VIVIEN K. WHITE.* *Dept. of Biological Chemistry, Harvard Med. School, Boston.*

Rat hemidiaphragms were incubated in duplicate Warburg vessels in a medium containing 11.1 mM/l. glucose and 10 mM/l, pyruvate; either the glucose or pyruvate was labeled. After a two-hour incubation, glycogen was isolated from the muscle, hydrolyzed to glucose and converted to glucosazone for counting. Aliquots of the medium were analyzed chemically for glucose, pyruvate, and lactate, and aliquots were precipitated as glucosazone and as pyruvate 2,4-dinitrophenylhydrazone and counted. The respiratory CO_2 was recovered from the center well, precipitated as $BaCO_3$ and counted. None of the glucose in the medium at the end of the incubation came from labeled pyruvate, chemical evidence that muscle has no phosphatase for glucose-6-PO_4. Five to 15% of the pyruvate isolated at the end of the incubation came from the labeled glucose. Seventy to 80% of the glucose and pyruvate disappearing from the medium was accounted for as lactate appearing, glycogen synthesized, CO_2 produced, and glucose converted to pyruvate. The amount of glycogen synthesized is increased when both glucose and pyruvate are present over that made when either is present alone. Insulin triples the percentage of glycogen made from glucose and decreases the percentage made from pyruvate to about one-third; but it causes so much more glycogen to be made that the total amount from pyruvate is the same or even increased. The data are in accord with the results obtained previously which indicate that the effects of insulin could be accounted for by a stimulation of the hexokinase reaction and an additional, lesser effect on the reactions involved in the condensation of pyruvate with oxalacetate to enter the citric acid cycle.

Photochemical reduction of pyridine nucleotides. WOLF VISHNIAC (introduced by S. OCHOA). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

Green grana of spinach leaves, prepared essentially as described by Warburg (*Schwermetalle als Wirkungsgruppen von Fermenten*, SAENGER, Berlin, 1948), were shown to reduce DPN and TPN in the light. The reduction of DPN was detected by the formation of lactate when pyruvate, DPN, and rabbit muscle lactic dehydrogenase were incubated with green grana in the light. Negligible amounts of lactate were found in the dark or in the absence of DPN or lactic dehydrogenase. The reduction of TPN was detected by the formation of *l*-malate from pyruvate and CO_2 in the presence of pigeon liver 'malic' enzyme (*J. Biol. Chem.* 174: 979, 1948). No malate was found in the dark or in the absence of TPN or 'malic' enzyme. Complete anaerobiosis and a pH of 7.0 were required for these reductions. The reduction produced the stoichiometric equivalent of O_2. The liberated O_2 was absorbed in acid $CrCl_2$ and determined by electrometric titration with I_2. These results show that the chloroplast preparations can catalyze the over-all reaction $2H_2O + 2DPN$ (or $2TPN$) $\xrightarrow{(light)}$ $2DPNH_2$ (or $2TPNH_2$) $+ O_2$. This reaction can then couple with $DPNH_2$ + pyruvate → lactate + DPN (lactic dehydrogenase) or $TPNH_2$ + pyruvate + CO_2 → *l*-malate + TPN ('malic' enzyme). The possibility that such a reaction mechanism might occur in photosynthesis has been previously suggested (*Federation Proc.* 9: 551, 1950; *J. Biol. Chem.* 187: 863, 1950).

Biological activity of substituted pyrimidine nucleosides. DONALD W. VISSER AND MARTIN ROBERTS (introduced by JOHN W. MEHL). *Dept. of Biochemistry and Nutrition, Univ. of Southern California, Los Angeles.*

Nucleosides in which bromine or chlorine are substituted for hydrogen on the number 5 carbon of the uracil moiety of uridine reversibly inhibit the growth of *Neurospora*, No. 1298, in the presence of cytidine or uridine. It was of interest, therefore, to synthesize other derivatives of uridine and cytidine and to test them in physiological systems. Accordingly 5-aminouridine was prepared by the ammonolysis of 5-bromouridine; 5-diazouridine by diazotization of 5-aminouridine, and 5-methylcytidine by the methods described by Todd *et al.* for the synthesis of cytidine. The

effect of these nucleoside derivatives upon the growth of *Neurospora*, No. 1298, and the propagation of mouse encephalomyelitis virus, Theiler's GD VII virus, in cultures of mouse brain will be discussed. Evidence will also be presented to indicate that in *Neurospora*, both uridine and cytidine are utilized as such, and the amination or deamination required for nucleic acid synthesis must occur at a subsequent stage of nucleic acid synthesis. (Contribution #280 from the department of Biochemistry and Nutrition; supported by Research Corporation.)

Glutamotransferase activity in mammalian tissue extracts. HEINRICH WAELSCH. *New York State Psychiatric Inst. and Dept. of Biochemistry, Columbia Univ., New York City.*

The presence of enzymes which catalyse the exchange of the amide group of asparagine and glutamine with other amines, e.g. hydroxylamine and hydrazine, has been demonstrated in cell-free extracts of microorganisms. The rate of enzymatic exchange (cell-free extract of Proteus vulgaris) is not influenced by the addition of phosphate, Mn^{++}, or mg^{++}. Tissue homogenates as well as cell free extracts of Proteus vulgaris promote the exchange of the amide group of glutamine with ammonia (isotopic), but the enzymatic formation of hydroxamic acids with hydroxylamine as acceptor amine could not be unequivocally demonstrated with extracts of mammalian tissues. Aqueous extracts of acetone dried powder of sheep brain cortex, liver, or kidney showed no glutamotransferase activity. However, the addition of manganous salts activated the transferase activity which was then considerably enhanced by the addition of phosphate. Phosphate alone without manganese did not activate the enzyme. Potent enzyme preparations were obtained from sheep and mouse organs as well as from a transplanted mouse sarcoma. Whereas homogenates of brain or kidney cortex possess strong glutaminase activity, extracts from the acetone dried powder of these organs, activated with manganese and phosphate for maximal transferase activity, were unable to split glutamine. This finding indicates that glutamotransferase activity is not associated with glutaminase in mammalian tissues.

Aureomycin and citrovorum factor in sulfa and aminopterin-induced folic acid deficiencies. HARRY A. WAISMAN, J. CRAVIOTO,* M. GREEN,* A. REMENCHIK* AND J. B. RICHMOND.* *Dept. of Pediatrics, Univ. of Illinois College of Medicine, Chicago.*

Levels of aureomycin, from 5–10 mg/100 gm. of purified diet, have proved to be completely effective in overcoming the toxic signs of 'folic acid' deficiency produced in rats when 75–100 μg. of aminopterin are included in the diet. This quantity of antibiotic was able to prevent the poor growth, hemorrhagic phenomena and death which occurred in weanling rats receiving the metabolic antagonist. Any attempt to attribute this action primarily to its antibiotic activity must reconcile the finding that intraperitoneal injection of aureomycin were also able to give growth despite the aminopterin. On the contrary, when aureomycin was autoclaved for 3 hours at a pH = 10, the compound was ineffective in overcoming the aminopterin-induced deficiency. A single daily intraperitoneal injection of both 5 μg. aminopterin and 5 mg. aureomycin to grown rats prevented the failure and death which would have resulted with the antagonist alone. Aureomycin was without influence on the deficiency induced by including 2% sulfaphthalidine in the diet. Citrovorum factor concentrates were more efficient than folic acid in overcoming both the effect of aminopterin and the deficiency produced on the sulfa diets. Vitamin B_{12} and ascorbic acid alone or in combination were unable to modify the ultimate course of the toxicity due to aminopterin and while ascorbic acid gave a transitory improvement on rats maintained for long periods on the sulfa diet, it was without any effect when given from the beginning.

Role of sulfhydryl groups in the bleaching and synthesis of rhodopsin. GEORGE WALD AND PAUL K. BROWN.* *Biological Labs. of Harvard Univ., Cambridge, Mass.*

The regeneration of rhodopsin after bleaching by light is blocked completely by the sulfhydryl reagent, *p*-chloromercuribenzoate (PCMB, 8×10^{-5} M). Monoiodoacetate and iodoacetamide (0.03 M) do not have this effect. The synthesis of rhodopsin from retinene$_1$ and rhodopsin-protein (opsin) also is completely blocked by PCMB. PCMB does not react with retinene$_1$, nor does it destroy rhodopsin. The inhibition of rhodopsin synthesis by PCMB is reversed in part by glutathione. Cysteine and glutathione condense spontaneously with retinene$_1$ at room temperature, apparently to yield complexes of the type: $C_{19}H_{27}CHO + RSH \rightarrow C_{19}H_{27}CHOH—SR$. The inhibition of rhodopsin synthesis by PCMB is directly proportional to the concentration of the reagent up to the point of complete inhibition. In our best rhodopsin preparations this point comes when about 6 mols PCMB have been added per mol retinene$_1$ contained in rhodopsin. The —SH content of rhodopsin solutions has been determined by amperometric titration with silver ions (Kolthoff and Harris; Benesch and Benesch). Before bleaching, such solutions reduce about 4 equivalents of silver ion per retinene equivalent. As rhodopsin is bleached to retinene$_1$ and opsin, two further —SH

groups appear for each retinene$_1$ molecule liberated. This is true equally of cattle, frog and squid rhodopsin. Sulfhydryl groups are therefore involved directly in the binding of carotenoid to protein in rhodopsin. In the amperometric procedure, the bleaching of rhodopsin produces a negative electrical variation which may be related fundamentally to the process of excitation in rod vision. (This research was supported in part by the Medical Sciences Division of the Office of Naval Research.)

Bacterial oxidation of pyrimidines. T. P. WANG* AND J. O. LAMPEN. *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

A motile, gram-positive, non-sporulating, rod-shaped bacterium has been isolated from soil by means of enrichment culture technique. It can grow aerobically on either uracil, cytosine or thymine as the sole source of carbon and nitrogen. Purines such as adenine and hypoxanthine support little or no growth. Sulfur analogs of uracil and thymine are not utilized. Resting cells of this organism oxidize uracil, cytosine or thymine to CO_2 and ammonia. The recovery of carbon and nitrogen is only about 65–75%. No evidence of assimilation was obtained, however, from experiments with 2,4-dinitrophenol. No fatty acids or lactate were found. The oxidation of substituted pyrimidines has been studied to evaluate the specificity of the action. Substituents on position 1 reduce slightly the rate of oxidation; on position 2 the hydroxyl group cannot be replaced by a hydrogen or an amino group; substituents on position 3 reduce the activity; substituents on position 4 (except —OH) destroy the activity completely; substituents on position 5 increase the activity; the hydroxyl group on position 6 can be replaced by an amino group. Cell-free extracts have been prepared from this organism. In the presence of methylene blue and air, uracil or thymine can be oxidized by the extract with an uptake of one atom of oxygen per mole of base. The product has been separated from the reaction mixture by an anion exchanger (Dowex-1). It has a sharp absorption peak near 260 mμ in neutral or alkaline solution and a much lower extinction in acid solution. The nature of this compound is under investigation.

Incorporation of orotic acid-2-C^{14} into pyrimidines of nucleic acid in vitro. LAWRENCE L. WEED* AND D. WRIGHT WILSON. *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

Orotic acid-2-C^{14} is converted to some extent into pyrimidine nucleotides in slices of surviving rat liver. All of the radioactivity of the nucleotides has been found in the pyrimidine bases. The study has been applied to slices of other tissues from these and other animals. The relative specific activities of uridylic and cytidylic acids of RNA and cytidylic and thymidylic acids of DNA have been examined.

Quantitative micro and macro analysis of cations and anions by flame photometry. T. E. WEICHSELBAUM, P. L. VARNEY* AND H. V. MARGRAF.* *Dept. of Surgery, Washington Univ. School of Medicine, St. Louis, Mo.*

Complete analytical data over a wide range of concentrations will be given on the mutual interference of sodium with the determination of potassium and of potassium with the determination of sodium. Methods of determining small amounts of calcium (less than 1 ppm) and magnesium (less than 10 ppm) will be described. The determination of anions by flame photometry will be illustrated by a method of determining sulfates. A comparative study of the spectral interference of certain cations using two different types of monochromaters will be presented. The question of the use of internal standards versus the use of fixed excesses of interfering elements will be illustrated. Concentration limits and interference data will be outlined for the following elements: Ba, Cs, Cu, La, Li, Mn, Pb, Sr, and Tl.

Metabolism of methylxanthines. HERBERT WEINFELD (introduced by A. A. CHRISTMAN). *Dept. of Biological Chemistry, Univ. of Michigan Med. School, Ann Arbor.*

Approximately 10% of caffeine injected intravenously into rabbits is found in the urine collected during the following 48-hour period. The urine, however, contained compounds which reduced alkaline phosphotungstates and were not hydrolyzed by uricase. Urine collected after feeding caffeine and theophylline to rabbits were studied by the methods of paper chromatography. After caffeine feeding, one of the reducing, uricase stable urinary compounds was demonstrated by this method to be 1,3-dimethyluric acid and evidence for the presence of 1-methyluric acid or 3-methyluric acid or a mixture of both was obtained. After feeding theophylline, a crude mixture of substances was isolated from the urine. Migration rate studies indicated that 1 methyl and 1-3 dimethyluric acid were present in the mixture. 3-methyluric acid was absent. Chromatography studies of human urine collected after oral administration of a caffeine revealed the presence of 1-methyluric acid. 1,3-dimethyl and 3-methyluric acids were not present in amounts which could be detected by this method.

Oxidation of endogenous fatty acid of rat tissues in vitro. S. WEINHOUSE, M. E. VOLK* AND R. H. MILLINGTON.* *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

The present study was undertaken to obtain information concerning the extent to which various tissues utilize fatty acids in respiration. Endogenous fatty acids of rat tissues were labeled by administering palmitic acid, labeled with C^{14} in the carboxyl group, to fasting rats by stomach tube. Three hours later various tissues were removed, and portions were taken for extraction, and analysis of the fatty acids for C^{14} activity. The remainder was sliced or minced and allowed to respire at 37° in oxygen for 4 hours, during which time respiratory CO_2 was collected. Analysis of the CO_2 indicated that all tissues of the rat, including liver, kidney, brain, spleen, heart and skeletal muscle utilize endogenous fatty acids for their respiration *in vitro*. Part of the endogenous palmitic acid of liver slices was converted also to β and carboxyl-labeled acetoacetate. With all tissues tested the specific activity of the respiratory CO_2 was higher (sometimes considerably higher) than the specific activity of the fatty acids. This finding suggests that there is a metabolically active fatty acid fraction which is not in rapid equilibrium with the bulk of the tissue fatty acids. No marked differences were found, however, between phospholipide and non-phospholipide fatty acids. By differential centrifugation of liver homogenates it was found that the microsome lipides had the highest specific activity followed by decreasing activities in the mitochondrial, nuclear, and supernatant fractions.

Formation of acetate from glycine. ARTHUR WEISSBACH* AND DAVID B. SPRINSON. *Dept. of Biochemistry, Columbia Univ., New York City.*

It has been shown that α-labeled glycine is converted in the rat to acetate labeled nearly equally in both carbon atoms (SPRINSON. *J. Biol. Chem.* 178: 529, 1949). The possible role of glyoxylate in this reaction was studied by administering 2-C^{14}-glyoxylate to rats in conjunction with benzoate or phenylaminobutyrate. The excreted glycine (as hippurate) had a high activity in the α-carbon and negligible activity in the carboxyl carbon. The acetate (from acetyl phenylaminobutyric acid) was almost equally labeled in both carbons. The utilization of glyoxylate for acetate formation was comparable to that obtained from glycine. Following administration of 2-C^{14}-glyoxylate to a pigeon carbon 5 of the excreted uric acid was six times as active as carbons 2 and 8 (cf. KARLSSON AND BARKER. *J. Biol. Chem.* 177: 597, 1949). These data suggest that glyoxylic acid is metabolized extensively via glycine. The conversion of the α-carbon of glycine to acetate or a one-carbon unit does not appear to involve glyoxylate as an intermediate. Administration of $HC^{14}OOH$ or $H_2C^{14}O$ afforded acetate in which the methyl carbon had 7–9 times the activity of the carboxyl carbon. 3-C^{14}-Serine has been shown to form acetate of approximately the same isotopic composition (ELWYN AND SPRINSON. *J. Biol. Chem.* 184: 465, 1950). It is possible that if the α-carbon of glycine is the precursor of a one-carbon unit, the latter condenses with glycine more rapidly than it equilibrates with other one-carbon intermediates, or that the one-carbon unit derived from glycine is capable of condensing with itself.

Synthesis of Pyo Ic. IBERT C. WELLS (introduced by W. W. WESTERFELD). *Biochemistry Dept., Syracuse Med. College, Syracuse, N. Y.*

Pyo Ic, an antibiotic isolated from *Pseudomonas aeruginosa* organisms (*J. Biol. Chem.* 159: 725, 1945), had an empirical formula of $C_{34}H_{48}N_2O_2$ based on a molecular weight of 486 as determined in tribromophenol by the Rast method. Ozonization of Pyo Ic gave N-caprylanthranilic acid as the major product (*Federation Proc.* 7: 198, 1948). The ultraviolet absorption spectrum of 2-methyl-4-quinolinol (*J. Am. Chem. Soc.* 68: 2181, 1946) is similar to that of Pyo Ic, and if they are related substances, Pyo Ic should be 2-nonyl-4-quinolinol. The synthesis and properties of this latter substance are now being reported. It was prepared from aniline and methyl-3-ketododecanoate following the procedure of Leonard *et al.* (*J. Am. Chem. Soc.* 68: 1279, 1946). 2-nonyl-4-quinolinol melts at 138–139°C. and does not depress the m.p. of Pyo Ic. The ultraviolet absorption spectrum is identical with that of Pyo Ic, and the solubilities and crystalline appearance of the two are qualitatively the same. Tetrahydro-2-nonyl-4-quinolinol melts at 156–157°C. and does not depress the m.p. of 'octahydro' Pyo Ic. These latter substances also have identical ultraviolet absorption spectra, and the solubilities and crystalline appearance are the same. The molecular weight of 2-nonyl-4-quinolinol is 506 as determined in tribromophenol by the Rast method, which indicates that a dimer is formed.

Cholinesterase activity in different spinal cord levels of the chick embryo. BYRON S. WENGER (introduced by OLIVER H. LOWRY). *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

Acetylcholine esterase activity of cervical, brachial, thoracic and lumbosacral levels of the spinal cord of chick embryos from 5½ to 11 days' incubation (Hamburger-Hamilton stages 28–37) has been measured with a new sensitive procedure based on the change in color of phenol red in a weak veronal buffer as acetic acid is liberated from acetylcholine. Individual samples (0.1–1 mg.) from given

spinal cord levels of single chick embryos are homogenized and aliquots representing as little as 3 μg. protein are assayed for cholinesterase activity. Protein determinations, and frequently additional enzymatic or chemical assays, are made on other aliquots of the same homogenate. In addition, data on wet and dry weights, protein and chloride are obtained from samples dissected in the cold after quick-freezing of the embryos. The period of development reported covers both the time of onset of nervous function as demonstrated by muscular response to stimulation and the later appearance of spontaneous activity. During the short period corresponding to the onset of nervous functions there is observed a five fold rise in cholinesterase activity (from 1.5–7 mM acetylcholine hydrolyzed per gm. protein per hour). Samples from the brachial and lumbosacral enlargements show generally higher activity than those from non-limb levels. An exception is found, however, at stages 33–34 where a more rapid increase in enzymatic activity is demonstrable in the thoracic level. (Supported in part by a USPHS (NIH) Research Fellowship and an American Cancer Society Grant.)

Application of infrared spectroscopy to the study of mercapturic acids. HAROLD D. WEST, NELSON FUSON,* MARIE-LOUISE JOSIEN,* ROBERT L. POWELL* AND LORETHA A. BLACK.* *Dept. of Biochemistry, Meharry Med. College and Infrared Spectroscopy Lab., Fisk Univ., Nashville, Tenn.*

In order to detect by means of infrared spectroscopy the presence of small amounts of mercapturic acids in extracts from the urines of animals fed certain aromatic substances, it was necessary to study the spectra of a number of model compounds. The models studied included several mercapturic acids. One of the mercapturic acids, S-*p*-chlorophenyl-N-acetyl cysteine, was synthesized for the first time *in vitro*. A discussion of its properties and a comparison with those of the same compound made *in vivo* will be given. It was possible to confirm the synthesis of the *p*-chlorophenyl mercapturic acid by infrared spectroscopic means before micro-analysis was completed because of the close similarity of its spectrum to that of the model compounds and in particular to other halogen substituted phenyl mercapturic acids. The spectra of the cysteines all showed typical amino acid absorption bands. The spectrograms in the 2–7 micron region of all the mercapturic acids studied have in common the absorption bands characteristic of amido acids. In addition each of these compounds shares to a striking extent the absorption profile, in the 8–15 micron region, of the foreign substance which produces the mercapturic acid. This work was supported by a grant from the Research Corporation.

Infrared spectroscopic studies of phenanthrene metabolism. HAROLD D. WEST, MARIE-LOUISE JOSIEN,* NELSON FUSON* AND ANDREW JACKSON.* *Dept. of Biochemistry, Meharry Med. College, and Infrared Spectroscopy Lab., Fisk Univ., Nashville, Tenn.*

Chang and Young (*Proc. Soc. Exper. Biol. & Med.* 53: 126, 1943) state that administration of phenenthrene to rats and rabbits results in an acid-decomposable precursor yielding free phenanthrene. No mention was made of the procedure which was used in the identification of the phenanthrene. In the course of a series of metabolism experiments carried out in these laboratories, 8 gm. of phenanthrene were fed to 3 dogs over a period of 7 days. The urines were extracted by a procedure similar to that used by Stroud (*J. Endocrinol. London* 2: 55, 1940) for isolation of certain metabolites, the 'combined neutral' fraction having the largest yield. Purification of this fraction gave a product consisting of flaky reddish crystals. The infrared spectrum of this metabolite was strikingly similar to that of pure phenanthrene, all important absorption bands being present in both spectrograms with the exception of a weak band at 5.95 microns in the spectrum of the metabolite but absent from that of phenanthrene. Presence of a trace of a quinone in the partially purified metabolite could easily account for the reddish color and for this extra band in the region of the spectrum where the absorption associated with the ring carbonyl is found. While this compound was not isolated in chemically pure form, there is, nevertheless, strong spectroscopic evidence for the presence of free phenanthrene as a metabolite resulting from the administration of phenanthrene. (This work was supported by a grant from the Research Corporation.)

Effect of diet on intestinal xanthine oxidase. W. W. WESTERFELD. *Biochemistry Dept., Syracuse Med. College, Syracuse, N. Y.*

The high starting level of intestinal xanthine oxidase in weanling rats (30 cmm. O_2/20 min/283 mg.) was depleted to a minimal value of 6 cmm. O_2 within one week by the feeding of a purified diet, irrespective of variations in casein content between 10 and 31%. The low value was maintained for at least 4 weeks. Similar results were obtained with a diet containing 10% liver residue but no casein (7% protein). The addition of 10% liver residue to a 21% casein diet maintained the intestinal xanthine oxidase at the starting value for 4 weeks, or restored it to such maximal values within 2 weeks when fed to depleted rats. Diets containing 21% casein plus 2% liver residue, or

10% liver residue plus 11 or 14% casein permitted the intestinal enzyme to fall to intermediate levels at 2 weeks, but gave a restoration to reasonably normal values by 4 weeks. Adult rats responded similarly but more slowly. Starvation caused a parallel loss of both liver and intestinal xanthine oxidases. The results showed that the deposition or maintenance of xanthine oxidase in the intestine was dependent upon a diet simultaneously adequate in both protein and the unidentified liver residue factor. Since the enzyme level in this tissue was not controlled to any greater extent by the protein than by the liver residue factor content of the diet, intestinal xanthine oxidase was far superior to the liver enzyme as a test object for the demonstration of this factor in the diet.

Utilization of 4-amino, 5-imidazole carboxamide for purine synthesis by yeast. WILLIAM J. WILLIAMS (introduced by JOHN M. BUCHANAN). *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Experiments were performed to determine the role of 4-amino, 5-imidazole carboxamide in purine metabolism in yeast. In one experiment carboxamide labeled with C^{14} in the 4 position was incubated for 4 hours with a suspension of a 16-hour culture of *Saccharomyces cerevisiae*. As a control, labeled sodium formate was incubated with a separate suspension of the same culture of yeast. In a second experiment labeled carboxamide was added to a rapidly growing culture of yeast and the culture allowed to grow for 10 hours. To control this experiment labeled glycine and sodium formate were added to two separate equal volumes of the same original culture. Following the incubation periods nucleotide adenine and nucleic acid adenine and guanine were isolated from the yeast and analyzed for radioactivity after purification. The results show that 4-amino, 5-imidazole carboxamide is converted to adenine and guanine by suspensions of yeast at a rate similar in magnitude to that of sodium formate (*experiment 1*). In the rapidly growing system (*experiment 2*) the purines formed from carboxamide were less radioactive than those formed from glycine and sodium formate. In this system approximately one mole of glycine was utilized for purine synthesis for every two moles of sodium formate. However, variations in this ratio occurred in certain instances and the significance of this variability will be discussed.

Mechanism of the growth-promoting effect of aureomycin in chicks. WILLIAM L. WILLIAMS,* ROBERT R. TAYLOR,* E. L. R. STOKSTAD AND THOMAS H. JUKES. *Lederle Labs. Div., American Cyanamid Company, Pearl River, N. Y.*

The growth-promoting action of aureomycin and other antibiotics when added to the diet of certain animals including chicks is presumably the result of an effect on the intestinal fluora. Microbial counts of the intestinal contents of chicks receiving a diet containing 100 mg. of aureomycin per kg. were compared with controls. The numbers of coliform-type bacteria and lactic acid bacteria were not significantly changed while the total numbers of anaerobic bacteria were reduced 150- to 200-fold by aureomycin. The clostridia-type anaerobes, about 1% of the total number of anaerobes, were decreased 10- to 150-fold. A high proportion of the clostridia produced large zones of hemolysis on blood agar plates. These hemolytic clostridia were eliminated almost completely from the intestinal contents and feces by feeding aureomycin. However, the combined antitoxins of *Clostridium perfringens*, type A, *C. septicum*, *C. histolyticum*, *C. tetani*, *C. novyi*, and *C. oedematoides* were without effect on the growth of control or aureomycin-fed chicks. Chicks on diets with and without aureomycin received oral and cloacal administration of combined sterile toxins or combined live cells of these organisms plus *C. fesseri* and 6 clostridia isolated from chick feces. The growth of these chicks was compared with that of uninoculated controls. The observations did not indicate a clear relation between the hemolytic clostridia and the growth of chicks.

Separation of saturated and unsaturated fatty acids by displacement chromatography. WILLIAM T. WILLIAMS* AND RALPH T. HOLMAN. *Dept. of Biochemistry and Nutrition, Texas Agricultural Exper. Station, College Station.*

Separations of unsaturated and saturated fatty acids of the same chain length have been achieved displacement chromatography using a modified Tiselius-Claesson adsorption analysis apparatus. Isolated double bonds in the fatty acid molecule decrease the adsorption of the acid. Alpha beta unsaturated acids are adsorbed more strongly than are other monoethenoic acids. Examples will be shown of separations in the C_4 series through the C_{18} series. Examples of the use of carrier displacement separations will also be given.

Metabolism of protein nitrogen during the healing of experimental wounds. MARTIN B. WILLIAMSON, THOMAS MCCARTHY AND HERBERT FROMM (introduced by W. R. TWEEDY). *Dept. of Biochemistry, Graduate School and Stritch School of Medicine, Loyola Univ., Chicago, Ill.*

It has been shown that wounds heal more rapidly when a high protein diet is supplied than when a low protein diet is given. When isocaloric diets containing 0.49% and 1.94% protein nitrogen (as casein) were fed to groups of 24 rats, the rate

of healing of experimental wounds, as measured by the increase in tensile strength (*Science* 105: 396, 1947), is greater in the animals fed the 1.94% protein N diet than in those on the 0.49% protein diet. However, the increase in tensile strength per total nitrogen available for healing was much greater on the low protein diet, even when there was a negative nitrogen balance, than on the high protein diet. This seems to indicate that more of the plasma and tissue protein nitrogen is being utilized for protein synthesis in the wound area on the low N diet than on the high N diet. Supplementing the low protein diet with gelatin so that the nitrogen content of the diet was the same as that of the high protein diet had little effect on the rate of healing of the wounds, but markedly increased the excretion of nitrogen. Supplementing the low protein N diet with gelatin and methionine so that it contained as much nitrogen and methionine as the high protein diet increased the rate of healing, and decreased the nitrogen excretion, to approximately the level found in the rats fed the high protein N diet. The rats on the low protein N diet retained a higher percentage of the dietary sulfur than did the animals on any of the other diets.

Reversal of tetraethyl pyrophosphate inhibition of acetylcholinesterase. IRWIN B. WILSON (introduced by G. L. FOSTER). *Dept. of Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*

Certain alkylphosphate esters such as TEPP and DFP have been considered irreversible inhibitors of acetylcholinesterase and certain other hydrolytic enzymes. Recently evidence has been offered that during the course of the enzymic hydrolysis of esters the basic group of the esteratic enzyme site is acetylated. The acetylated intermediate reacts with nucleophilic reagents to regenerate the enzyme and yield acylated products. Similarly during alkylphosphate inhibition the basic group is phosphorylated. This phosphorylated enzyme also reacts with nucleophilic reagents to regenerate the enzyme. Thus TEPP inhibition can be reversed slowly by reaction with water and rapidly by reaction with hydroxylamine and choline. If the reaction with water were rapid, TEPP would be a substrate rather than an inhibitor.

Fecal fatty acids and other lipids: A study of two normal human adults taking (*1*) a diet free of lipid and (*2*) a diet containing triolein as the only lipid. ERIC E. WOLLAEGER,* WALTER O. LUNDBERG, JACQUES R. CHIPAULT* AND HAROLD L. MASON. *Hormel Inst., Austin, and Mayo Fndn., Rochester, Minn.*

The fecal lipids of two normal men were measured during 4 successive dietary periods. In the first and fourth periods, the men received a general mixed diet; in the second, a lipid-free diet; and in the third, a diet of the same composition as that used in *period 2* except that 100 gm. of triolein per day was substituted isocalorically for some of the carbohydrate in the lipid-free diet. Feces were analyzed for dry matter, total lipids, and cholesterol. The component saturated, monoenoic, dienoic, trienoic and tetraenoic acids of the lipids were measured. Melting points, and neutralization equivalents of the total fatty acids were determined. When the lipid-free diet was substituted for the mixed diet containing fat, the excretion of total lipids dropped from an average of 7.7 gm/day to 1.5 gm/day, and the excretion of total fatty acids dropped from 4.3–0.45 gm/day. There also was evidence of a difference in the composition of the total fecal fatty acids while the men were on the two dietary regimens. During the triolein period, the fecal material yielded excessive amounts of component fatty acids, not all of which were monoenoic. The composition of the plasma fatty acids did not resemble that of the fecal fatty acids.

Substrate specificity of rabbit polymorphonuclear leucocyte ali-esterase. ESTHER WONG (introduced by A. B. MACALLUM). *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*

The effect of changing the number of carbon atoms in either the acyl group or the alkyl group on the rate of hydrolysis of a series of straight chain aliphatic esters by human red cell true cholinesterase, human plasma pseudocholinesterase and a typical ali-esterase was studied. The ali-esterase was the ester splitting enzyme of rabbit polymorphonuclear leucocytes previously described from this laboratory (*J. Biol. Chem.* 180: 933, 1947). In confirmation of the work of Adams and Whittaker, (*Biochim. et Biophys. Acta* 3: 1, 358, 1949) the optimal number of carbon atoms in the acyl group was found to be 2 (i.e. acetyl esters) for the true cholinesterase and 4 (i.e. *n*-butyryl esters) for the pseudocholinesterase. For the rabbit polymorphonuclear leucocyte ali-esterase the optimal number of carbon atoms in the acyl group was either 5 (i.e. *n*-valeryl esters) or 6 (i.e. *n*-caproyl esters). Also, in confirmation of Adams and Whittaker, the optimal number of carbon atoms in the alkyl group was found to be 4 (i.e. *n*-butyl esters) for both the true cholinesterase and the pseudocholinesterase. Changes in the number of carbon atoms in the alkyl group had less effect on the rate of hydrolysis of esters by the ali-esterase. The true cholinesterase and the pseudocholinesterase both hydrolyzed iso-amyl esters more rapidly than the corresponding *n*-

butyl ester, whereas the reverse was true for the ali-esterase.

Fixation of CO_2 in acetic acid by *Clostridium thermoaceticum*. HARLAND G. WOOD AND D. L. HARRIS.* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Clostridium thermoaceticum ferments glucose with almost a quantitative yield of acetate and Barker and Kamen have found C^*O_2 to be fixed in both the methyl and carboxyl carbons in almost equal amounts. They proposed that the fermentation occurs as follows: $C_6H_2O_6 + 2\ H_2O \rightarrow 2\ CH_3{\cdot}COOH + 2\ CO_2 + 8\ H$; $2\ C^*O_2 + 8\ H \rightarrow C^*H_3\ C^*OOH$. It remained possible that the C^*O_2 was not fixed in adjacent carbons ($C^*H_3{\cdot}\ C^*OOH$) but the equal concentration of tracer was a consequence of two processes yielding a mixture of $C^*H_3{\cdot}COOH$ and $CH_3{\cdot}\ C^*OOH$. Although it is impossible by chemical means to differentiate between these mixtures of molecules it is possible to do so by mass spectrometer analysis. The acetate formed from $C^{13}O_2$ and glucose was converted to ethylene and the relative abundance of $H_2C^{13}=C^{13}H_2$, $H_2C^{12}=C^{13}H_2$ and $H_2C^{12}=C^{12}H_2$ was determined. A substantial amount of $H_2C^{13}=C^{13}H_2$, mass 30, was observed. However, the fixation occurred not alone by doubly labeling since there was a high concentration of $C^{12}H_3{\cdot}\ C^{13}OOH$. Evidence has been obtained with carboxyl and methyl labeled acetate that acetate is not a stable end product. The carboxyl group of acetate exchanges with CO_2 during the glucose fermentation and it may be this reaction by which CO_2 is fixed in the carboxyl group. The validity of the mass analysis method has been shown with synthetic C^{13}-ethylene.

Structure-activity-relationships among analogs of dimethyldiaminobenzene as growth-inhibitors. D. W. WOOLLEY AND A. PRINGLE.* *Rockefeller Inst. for Med. Research, New York City.*

Because, 1,2-dichloro-4,5-diaminobenzene, an analog of 1,2-dimethyl-4,5-diaminobenzene, has been shown to function as an antimetabolite of this precursor of riboflavin and of vitamin B_{12}, a further study of relatives of this metabolite has been undertaken. The dichloro-compound was a selectively toxic agent which affected only species requiring riboflavin and B_{12} nutritionally. Furthermore, these 2 vitamins were unable to counteract the analog. Many structural relatives of the dimethyldiaminobenzene were synthesized, several of them for the first time, and tested for potency against *Staphylococcus aureus*, and mice. Alkylation of one amino-group, or replacement by nitro, abolished activity even when one or both of the methyl-groups was simultaneously exchanged for chlorine atoms. When one hydroxyl replaced one amino, active agents were formed, either when the methyls were untouched, or when they were exchanged for chlorines. Alkylation of the hydroxyl abolished activity. The nitrophenols formed by replacing the amino groups with hydroxyl and nitro were very potent, provided that at least one of the methyls was also exchanged for halogen. When the competitive antagonism with the diaminodimethylbenzene was tested, a similar relationship to that already established with analogs of phenylalanine (chloromycetin, etc.) was found. The activity of the aminophenols was only partially reversed by the metabolite, and that of the nitrophenols not at all. Halogenation increased the tendency to non-competitive behaviour with the metabolite. Nevertheless, the amino- and nitro-phenols still actively inhibited the biosynthesis of B_{12}, just as the dichlorodiamine has been shown to do in growing bacterial cultures.

Effect of lanthanum upon *Streptococcus faecalis* R. M. WURM (introduced by KURT G. STERN). *Div. of Neoplastic Diseases, Montefiore Hosp., New York City.*

Lanthanum is known to react with nucleic acids to form insoluble complexes. Therefore, its effect upon a rapidly multiplying organism with known nutritional requirements was studied. Although *S. faecalis* was unable to multiply in basal media containing excess La^{+++}, this resulted from a $PO_4^{=}$ deficiency due to precipitated $LaPO_4$. Inorganic $PO_4^{=}$ not only is an essential metabolite, but small increments (.002 mEq.) induce quantitative growth responses. In a phosphate-free medium, however, heavy bacterial suspensions produced measureable quantities of lactic acid and this resting metabolism was depressed by La^{+++}. Growing cultures containing equal excesses of $PO_4^{=}$ showed progressively lower acid titres with increasing concentrations of $LaPO_4$. This inhibition was apparently caused by La^{+++} resulting from the partial solubility and dissociation of $LaPO_4$. When the number of bacteria was controlled by varying the amount of *dl*-isoleucine or $PO_4^{=}$ in the medium and growth occurred in the presence of equal amounts of $LaPO_4$, containing radioactive La^{140} as tracer, approximately uniform amounts (6×10^{-4} mEq.) of lanthanum were taken up by all the cultures. Estimates based upon the amount of lanthanum recovered from supernatants of cultures in which no growth occurred indicate a solubility product of $LaPO_4$ of approximately $1.7\text{–}2.4 \times 10^{-8}$. When cells were exposed to uniform concentrations of lanthanum in a phosphate-free medium or in isotonic saline, the amount of La bound was proportional to the number of cells present. Similarly, when equal aliquots of heavy cell suspensions were exposed to

varying amounts of La^{+++}, the amount bound was directly proportional to the La concentration. (Work performed under contract AT-30-1-880 with the U. S. Atomic Energy Commission.)

Potassium clearance in renal dysfunction. QUENTIN YOUNG,* ARTHUR BERNSTEIN,* LIONEL BERNSTEIN,* WILLIAM ROSE* AND WILLIAM S. HOFFMAN. *Hektoen Inst. for Med. Research, Cook County Hosp., and Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Potassium clearances were determined before and during 74 specific renal function tests in patients with Bright's disease (*group 1*), during recovery from lower nephron nephrosis (*group 2*), in patients with hypopotassemia, chiefly alkalosis and diabetic acidosis (*group 3*). Serum potassium levels ranged between 3.4 and 5.6 mEq. per liter in *group 1*. Patients with higher levels were excluded from tests because of severe oliguria. Potassium excretion rates showed no correlation with C_M, C_{PAH}, or Tm_{PAH}, but did with urine flow. When flow exceeded 1.5 cc. per minute, projected potassium excretion exceeded 40 mEq. per day in 10 of 11 instances. When urine flow was below 1.0 cc. per minute, potassium excretion was less than 40 mEq. per day in 8 of 10 subjects. Potassium excretion remained high even with markedly reduced renal function so long as oliguria was absent. With anorexia or vomiting, negative potassium balances could be anticipated. Excessively high potassium clearances in spite of serum potassium levels from 1.8 to 3.6 mEq. per liter occurred in alkalosis and healing lower nephron nephrosis, possibly because of distal tubular damage. Tubular secretion of potassium was manifest in 18 experiments in which C_K exceeded C_M. If tubular potassium secretion is related to T_M, it normally must be considerably high. Its site appears to be the proximal convoluted tubule.

Role of ammonia in anaerobic nitrogen fixation. I. ZELITCH,* R. H. BURRIS AND P. W. WILSON. *Depts. of Biochemistry and Agricultural Bacteriology, Univ. of Wisconsin, Madison.*

It was observed earlier that *Clostridium pasteurianum* cells supplied N^{15}-N_2 excreted free ammonia with a phenomenally high enrichment of N^{15} into the medium. To investigate further the metabolism of ammonia by *C. pasteurianum*, cultures that had been grown on N_2 were given $N^{15}H_3$ and normal N_2. There was an immediate assimilation of N^{15} and an increase of N^{15} in the cells that was linear with time. The fact that this nitrogen fixing organism can assimilate ammonia without a period of adaptation suggests that ammonia is a normal intermediate in nitrogen fixation. The N^{15} concentration of the ammonia in the supernatant and soluble portion of the cells decreased at a rapid rate; the added $N^{15}H_3$ equilibrated with a source of readily available ammonia in the supernatant and soluble portion of the cells. This occurred also when H_2, instead of N_2, was passed through the culture supplied $N^{15}H_3$. The distribution of N^{15} in *C. pasteurianum* given $N^{15}H_3$ for a short time was investigated. The amino acids and ammonia were separated on a Dowex 50 column. Glutamic acid and ammonia had the highest concentration of N^{15} in the cells. The overall distribution of N^{15} in the amino acids was very similar when *C. pasteurianum* cells were supplied N^{15}-N_2 and when supplied $N^{15}H_3$. These data support the hypothesis that ammonia is a key intermediate in biological nitrogen fixation.

Enzymic degradation of guanidines by *Mycobacterium smegmatis*. E. A. ZELLER, L. S. VAN ORDEN AND W. F. KIRCHHEIMER (introduced by L. S. FOSDICK). *Depts. of Chemistry and Bacteriology, Northwestern Univ. Med. School, Chicago, Ill.*

Previous studies indicated that streptomycin acts strongly and directly on bacterial diamine oxidase, presumably through its guanidine groups (ZELLER, E. A., C. A. OWEN, AND A. G. KARLSON. *J. Biol. Chem.* In press). In connection with these studies the question arose as to whether enzymes which are able to attack guanidines can also be blocked by this antibiotic through a similar mechanism. Since little is known about the enzymic degradation of guanidines, this process was investigated in general and with particular consideration to mycobacteria. Streptomycin-sensitive as well as resistant and dependent strains of *Mycobacterium smegmatis* were found to deaminate tetramethylene diguanidine and agmatine, but not methylguanidine, creatine and L-arginine. The investigation was complicated by the fact that more than one enzyme is involved in the complete deamination of the guanidines, and by the presence of a very strong urease in this microorganism. The enzyme responsible for the first step in the degradation of guanidines will be characterized. It is, as well as the urease, much less sensitive to streptomycin than diamine oxidase.

Effects of an anterior pituitary extract and thyrotropin on renal excretion of administered radiophosphorus. E. M. ZORN,* W. R. TWEEDY AND G. V. LEROY.* *Radioisotope Unit, Veterans Administration Hosp., Hines, Ill.*

Rats weighing 160–220 gm. were placed on a diet (KLEIBER, BOELTER AND GREENBERG. *J. Nutrition* 19: 517, 1940) containing 10 mg. of Ca/100 gm., but no added P, for 2–3 weeks. During the following 8 days, while still on this diet, each experimental animal received by subcutaneous injection 75 mg. (solid content) of an alkaline

extract of whole anterior pituitary gland powder (Armour) in 10 equal amounts. When the last dose was given, each animal and its control was injected intraperitoneally with 15 μc. of essentially carrier-free P^{32} and the urine collected during the next 5 hours. The 26 experimental animals excreted 4.4 ± 2.2% of the dose of P^{32}, while the 26 controls excreted 1.5 ± 0.9% of the dose. The S.E.D. of the two mean values was found to be 0.5. In later experiments in which rats were placed on the above diet, a total of 7.5 mg. of thyrotropin (Armour) in 10 equal amounts, was injected. The 25 rats injected with this preparation excreted 3.0 ± 2.3% of the dose of P^{32} in the urine, while their controls excreted 2.4 ± 1.4% of the dose. The S.E.D. of these mean values was also found to be 0.5. Variations in the normal histology of the thyroid or parathyroids were present in both the experimental animals and their controls and are attributed to the effects of the diet alone.

Influence of age on mouse liver phosphatases. ANITA ZORZOLI (introduced by E. S. HILL). *Dept. of Biochemistry, Washington Univ. Dental School, St. Louis, Mo.*

The acid and alkaline glycerophosphatase activities of the liver of C 57 Black (subline 6) mice are being studied as a function of the growth and aging of the animals. These quantitative data are being correlated with studies of the histochemical localization of the enzymes with the same tissues. With the exception of the early stages where several livers were pooled, the data from each age group represent the analysis of individual livers from equal numbers of males and females. To date a total of 90 animals have been studied. Preliminary studies were made to determine the optima of pH and substrate concentration and the influence of glycine and Mg ions. Glycine had no significant activating effect on the alkaline glycerophosphatase whereas $MgCl_2$ in a 0.01 M final concentration produced a 10- to 20-fold increase in activity. Both the acid and alkaline phosphatases had significantly higher activities in 2-, 10-, 15- and 20-day-old animals than at any older age. Starting at the 27th post-natal day there was a 50–60% decrease in activity of the two enzymes and from this time to 1 year of age (studied at monthly intervals) the activity of the two enzymes remained remarkably constant both within each age group and between age groups. The total nitrogen values, on the other hand, were constant from the 2nd postnatal day to 1 year of age, indicating a true enzyme activity difference. Work is now in progress on fetal stages and additional young animals.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

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(An asterisk following an author's name indicates "by invitation")*

Refractory period and conduction time studies with Ethyl Ether, Cyclopropane and Chloroform using turtle ventricle strips. JOHN ABAJIAN, JR. AND EDWARD BRAZELL (introduced by N. B. DREYER.) *Dept. of Anesthesiology, Univ. of Vermont, Burlington.*

Certain hydrocarbons are more predisposing than others to sudden death from ventricular fibrillation. Statements in the literature such as 'sensitizing the automatic tissues of the heart' are not too definitive. Shortening of the refractory period and prolongation of the conduction time is said to set the stage for re-entry. Studies were undertaken using turtle ventricle strips in a chamber filled with Ringer's. Isometric contraction was recorded with a strain gauge. Electromyogram was recorded simultaneously with platinum electrodes, the indifferent electrodes placed distally in the chamber. The first pick-up electrode was placed close to the non-polorizable stimulating electrodes; the second, one centimeter down. Recording was done with direct coupled amplifiers and direct coupled twin-beam cathode ray oscilloscope. The stimulator was a series of multi-vibrators producing square waves of varying amplitudes, widths and repetition rates which were fixed at any desired driving rate; whenever desired another variable square wave could be interpolated following the initial stimulus. The refractory period measured mechanically and by the Q.T. interval of the electromyogram. Conduction time was derived by the time necessary for propagated impulse to travel from the first to the second electrode pick-ups. The end point for comparison was arbitrarily selected as the 50% reduction of initial tension. At this point, Ethyl Ether revealed the shortening of the refractory period by 20%, Cyclopropane 12%, Chloroform 3.7%. The conductive time revealed no change in the case of Ethyl Ether or Cyclopropane but approximately 50% lengthening with Chloroform.

Influence of 4*n*-alkoxy benzilic acid esters of diethylaminoethanol upon canine tremor-rigidity syndrome. BENEDICT E. ABREU, ALICE B. RICHARDS,* JOSEPH M. LEAP* AND WILLIAM M. ALEXANDER.* *Research Dept., Pitman-Moore Company, Indianapolis, Ind.*

McGovern *et al.* (*Med. J. Australia* 2: 492, 1948) have noted the physical similarity existing between the canine tremor-rigidity syndrome and paralysis agitans in man. This has suggested the possible utility of dogs afflicted with this syndrome in evaluating potential 'anti-parkinsonism' drugs. Dogs were selected in which signs had not progressed for a period of 6 months, although it has been found entirely feasible to use animals which have shown signs for as little as 3 months, since they are usually stabilized at this time. Two dogs each received 5 mg/kg., i.v. of the straight chain members of the series which were available. Abolition of signs (+), duration in minutes, and skeletal muscle weakness and ataxia (1+–4+) were recorded and are indicated in the table.

INFLUENCE OF 4 *n*-ALKOXYBENZILATES UPON THE CANINE TREMOR-RIGIDITY SYNDROME

DRUG	SIGNS ABOLISHED	DURATION (MINUTES)	SKELETAL MUSCLE WEAKNESS AND ATAXIA
ethoxy	+	43	4+
n-propoxy	+	42	2+
n-butoxy	+	93	1+
n-amoxy	+	98	1+
n-hexyloxy	+	52	1+
n-heptyloxy	0	0	1+
n-oxtyloxy	0	0	1+

Adrenergic blocking agents. RAYMOND P. AHLQUIST AND JAMES P. TAYLOR.* *Dept. of Pharmacology, Med. College of Georgia, Augusta.*

The effects of the adrenergic blocking agents were studied on the mean arterial pressure of anesthetized, atropinized dogs by infusing these agents in balanced amounts while the animal's blood pressure was being maintained at hypertensive levels by automatically controlled infusions of epinephrine or arterenol. Two screw-driven syringes, each actuated by a continuous stepping relay and electrical metronone, were used to inject the pressor agent and the blocking agent. The arterial pressure of the animal automatically controlled the drug injections by means of an electronic-relay-barostat. When the pressure was below a preset level (usually 10–50 mm. Hg above the control pressure) the pressor agent was injected;

when above this level the blocking agent was injected. Since the barostat was of the off-on type one or the other drug was always being injected. The amounts injected were read from calibrated counters at intervals of 5 or 10 minutes. The following could be demonstrated by this method: *1*) the minimal adrenergic pressor blocking dose, *2*) the rate of onset and the duration of significant adrenergic blockade, and *3*) the influence of adrenergic activation on adrenergic blockade. Detailed results with several blocking agents representing various chemical types will be presented. (This work was supported by a grant from the American Heart Association, Inc.)

Method for evaluating actions of drugs on bronchiolar resistance. WILLIAM M. ALEXANDER* AND BENEDICT E. ABREU. *Research Dept., Pitman-Moore Company, Indianapolis, Ind.*

Previously employed technics which were considered useful for indicating changes in bronchiolar resistance (see D. E. Jackson method and modifications) have been considered undesirable because of the extensive surgical intervention necessary and resulting unphysiologic conditions. The method employed involves the recording of inspiratory stroke volume by means of a Krogh spirometer, while inspiratory efforts of the diaphragm are maintained constant using the electrophrenic technic of Sarnoff *et al.* (Kindly suggested by Dr. Gordon A. Alles during a visit to this laboratory). Dogs anesthetized with pentobarbital sodium were used throughout, and after a control period, representative bronchiolar depressants, e.g., epinephrine, isopropylarterenol, atropine, papaverine, theophylline sodium glycinate and diphenhydramine were injected intravenously. These all produced a slight increase in inspiratory stroke and minute volume, even though bronchoconstriction had not been induced by previous administration of agents known to induce such effects. Following this, representative bronchoconstrictors, e.g., histamine, acetylcholine, neostigmine and carbachol, were administered and produced a prompt reduction in inspiratory stroke and minute volume. Bronchoconstrictor-bronchodilator antagonism was best quantitated by: *1*) simultaneous injection of constrictor and dilator, and by varying ratios of each or *2*) treatment with a dilator and a challenge with increasing constrictor drug dosage. Antagonism was not as quantitatively reproducible when the constrictor was given prior to the dilator.

A new method for the estimation of gentisic acid. EDWARD L. ALPEN AND VICTOR W. RODWELL (introduced by PAUL K. SMITH). *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

Because of the present interest in gentisic acid as an agent in the treatment of rheumatic fever a sensitive method for its estimation is desirable. Previous methods suggested have been the use of the Folin-Ciocalteau phenol reagent and the hydroquinone reaction of Fassett (*Federation Proc.* 8: 90, 1949). It was found that both methods failed to differentiate gentisic acid from other normal urinary constituents. When diazotized sulfanilic acid is added to a solution of gentisate to which sodium carbonate has been added a yellow color develops. This yellow color is converted to pink when coupling is stopped with 3N sodium hydroxide and hydroxylamine solution is added. This pink color has a maximal absorption at 525 mμ and follows Beer's law for concentrations up to one mg. % in the total reaction mixture. A strong yellow color is developed in blank urines, but it is not converted to pink by hydroxylamine and alkali. The interference can be completely eliminated by reading optical densities at 575 mμ. The precision of measurement is improved by the use of a Corning 3060 filter. It was found that the diazotized sulfanilic acid, which was made up daily, should be allowed to stand 30 minutes before use, and that it should be checked against a standard gentisate solution.

Comparative toxicity of pyrethrins and allethrin. ANTHONY M. AMBROSE AND DOROTHY J. ROBBINS.* *Pharmacology Lab., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.*

Purified pyrethrins containing 86.2% total pyrethrins and synthetic allethrin containing 93.6% allethrin administered gastrically or subcutaneously to rats in doses of 2.6 and 1.6 gm/kg., respectively, produced no toxic reactions. When rubbed into the skin of rats in amounts of 50 mg. daily for 30 days, they produced no local reactions. On albino guinea pigs and on the anterior cubital surface of three humans, purified pyrethrins or allethrin produced no signs of local irritation. With less pure allethrin some transitory skin irritation was observed. Albino guinea pigs were not sensitized by topical application or intracutaneous injection of purified allethrin. The effect of prolonged oral ingestion of one sample of commercial allethrin and a purified sample of commercial allethrin, assaying 72.4 and 92% allethrin, respectively, was studied in rats on diets containing 78, 156, 312, 625, 1225, 2500 and 5000 ppm of the respective allethrin samples. Rats on the diet containing 5000 ppm commercial allethrin showed a slight decrease in growth as compared with rats on the diet containing the same concentration of purified allethrins. Hematological findings on rats on the various dietary levels of the two allethrin samples

were indistinguishable from those of the controls. From these preliminary observations, it is concluded that purified allethrin is no more toxic than purified pyrethrins. The toxic reaction observed after topical application or after prolonged oral ingestion is undoubtedly due to impurities. More extensive studies on chronic toxicity are in progress.

Tolerance of a vitamin C-sulfone derivative in man. HAMILTON H. ANDERSON, ALEXANDER N. CONTOPOULOS,* CHARLES NUCHO* AND GREGORY HADDAD.* *American Univ. of Beirut and Hamlin Memorial Sanitorium, Hammana, Lebanon.*

A crystalline derivative of bis (4 amino-phenyl)-sulfone synthesized by condensation with vitamin C by P.P.T. Sah *et al.* (*Recueil des Travaux Chim. des Pays-Bas* 68: 118, 1949) has been studied in mice and rats by Bierwagen *et al.* (*Proc. Am. Soc. Pharmacol. & Exper. Therap.*, 1950). In leprous infected rats, 0.5% in the diet given daily over 4–6 months was tolerated, without appreciable toxicity except the occurrence of slightly enlarged spleens and a fall in hemoglobin level (<10% Sahli). An extension of these studies has been made in adult tuberculous patients under sanatorium conditions. In 11 persons (1 female, 10 males) given total dosage up to 52.5 gm. over periods up to 60 days, the following changes were noted. The hemoglobin level was reduced (from 13–20%) in 3, while in 1 other it was elevated (25%). Total leucocyte counts were increased in 5, while in none was there a significant decrease. Liver and kidney function tests did not reveal evidence of impairment. Albumen appeared in the urine of 3 persons, granular casts in 1 other, and sugar in 1. Grains in body weight (from 1.0–3.0 kg.) were noted in 5 of the 11 under study. Untoward reactions, believed to be due in part to drug administration, occurred in 3 patients. Fever, nausea, vomiting and abdominal distension were of sufficient severity to cause discontinuation of the drug in 2 persons. (Supported in part by the Lilly Research Laboratories, Indianapolis, Indiana.)

Effects of procaine on histamine actions and histamine release. JOHN D. ARCHER,* JOE B. NASH,* J. EUGENE HILL* AND PAUL L. EWING. *Univ. of Texas Med. Branch, Galveston.*

Results of procaine administration in treating serum sickness and other conditions in which histamine release is a factor suggest that procaine may antagonize histamine actions or release. In confirmation of Code, no evidence of these actions was found in sensitized rabbits after challenge doses of Freund antigen, or in normal rabbits and man, using skin tests and systemic reactions as indicators. Procaine, in high concentrations, interferes with the assay technique of Barsoum and Gaddum, but does not do so at levels resulting from therapeutic doses.

Duration of therapeutic plasma penicillin levels with the penicillin salt of 1,2-diphenyl-2-methylaminoethanol. M. C. BACHMAN AND V. YOUNG (introduced by F. H. SCHULTZ, JR.). *Pharmaceutical Research Div., Commercial Solvents Corp., Terre Haute, Ind.*

A number of repository penicillin salts have been prepared and evaluated, using procaine penicillin as a standard. Of the group studied, the penicillin G salt of 1,2-diphenyl-2-methylaminoethanol was found to be most promising. This material, as prepared in this laboratory, has a penicillin potency of 1050 U per mg., is soluble to the extent of 240 mg. in 100 ml. of distilled water at 25°C., and is stable in the dry form at 100°C. for 96 hours. Experiments were performed to test the ability of this new penicillin salt to protect animals against experimentally produced infections, using procaine penicillin as a standard. Groups of normal mice were given a single intramuscular injection of this new penicillin salt, in a dose of 15,000 U, suspended in water, vegetable oil, or oil with 2% aluminum monostearate. A virulent culture of *D. pneumoniae* (10,000 MLD's) was injected into some of these mice one hour after they had received penicillin; other mice were infected at 12 and 24 hours following penicillin administration; and the remaining mice received the pneumococci 48, 72, 96, 120 and 144 hours after administration of the penicillin salt. A parallel series with procaine penicillin was run simultaneously. Results of these studies show that the penicillin salt of 1,2-diphenyl-2-methylaminoethanol gives therapeutic penicillin levels of longer duration than does the procaine salt, when each is in aqueous or oil formulation. Nearly identical results were obtained when both salts were formulated in oil with 2% aluminum monostearate.

Irritant effects of the hygroscopic agent in cigarettes as shown by electroencephalography. T. C. BARNES. *Dept. of Pharmacology, Hahnemann Med. College and Hosp. of Philadelphia, Philadelphia, Pa.*

Mulinos (*Proc. Soc. Exper. Biol.* 32, 1934) and Wallace, (*Arch. Otolaryngology* 23: 306, 1936) have shown that smoke from cigarettes containing glycerine as hygroscopic agent is more irritating to the eye of rabbits than smoke from cigarettes containing diethylene glycol. By the use of the electroencephalograph (EEG) their conclusions have been confirmed in man. A 6-channel EEG was used, recording EEG, pulse rate, palmar sweating (psychogalvanic reflex), action currents from muscle and eye-brow movements. Persons tested kept eyes closed and were not aware of the type of cig-

arette smoked. The sequence of the type of cigarette was altered to eliminate accumulative effects. Before each cigarette was ignited, it was puffed unlit, including buccal and nasal exhalation, in imitation of actual smoking. Most subjects showed EEG changes from the unlit cigarette, the effects being used as a correction factor in determining the effects from smoking. The percentile time occupied by the normal relaxation waves is designated as the alpha index. This was 56% for diethylene cigarettes and 41% for the glycerine cigarettes. This difference indicates a greater irritation from smoke of glycerine cigarettes than from smoke of di-ethylene glycol cigarettes. In 127 EEG recordings, pulse rates above 90 occurred 60% with the glycerine cigarettes and 41% with the diethylene glycol cigarettes showing rapid heart rate produced by the greater irritation of the smoke of cigarettes containing glycerine.

Models demonstrating the electrical action of atropine on cellular receptors. T. C. BARNES. *Dept. of Pharmacology, Hahnemann Med. College of Philadelphia, Philadelphia, Pa.*

Previous reports (*Science* 110: 511, 1949; *J. Pharmacol. & Exper. Therap.* 98: 2, 1950; *Med. Physics* 2: 80, 1950) have described the phase-boundary potentials of drugs. A model of drug receptors consists of a glass cell 20 mm. by 10 mm. having 3 circular apertures 1.7 mm. in diameter which can be opened or closed. The cell can be connected to larger receptors 19 mm. and 46 mm. in diameter. Since 'membrane' potential is independent of area, it is unnecessary to cover the entire surface of a cell with drug molecules. Thus 25 mg. of atropine produced 20 mv. negativity when added to 200 cc. of saline in contact with a guaiacol receptor 19 mm. in diameter. This atropinized receptor blocked the action of 25 mg. of acetylcholine which produced only 2 mv. negativity. Identical results were observed when atropine in the same concentration blocked a receptor of 46 mm. diameter or one or two receptors each 1.7 mm. in diameter. A blocked 1.7 mm. receptor was renewed by the injection of fresh oil but the atropine block returned in 4 minutes. The 'inert' sodium carboxymethylcellulose (Carmethose, Ciba) produced a large positive potential. 225 mg. Carmethose in 200 cc. saline generated 160 mv. positivity which modified the negative potentials of acetylcholine and atropine. These effects may be factors in the treatment of peptic ulcers with Carmethose. Atropine and acetylcholine had no effect on adrenergic (triglyceride oil) receptors in contrast to 0.05% isopropylarterenol which produced 15 mv. negativity on tributyrin.

Trasentine analogues—inhibitors of gastric secretion. WALTER BARRETT,* RICHARD RUTLEDGE,* ALBERT J. PLUMMER AND FREDERICK F. YONKMAN. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Certain members of a large series of Trasentine derivatives (R. MEIER AND K. HOFFMANN. *Helvet. Med. Acta* 7: 106, 1940/41) were extensively studied for therapeutically significant pharmacologic activities. The antacid and antigastric secretory properties were studied by the Shay and Visscher rat procedures and in dogs with gastric fistulae and Pavlov pouches. Of all compounds examined, C-5473 (phenylcyclohexyl-oxyacetic acid-diethylaminoethylester-brommethylate) was the most active in inhibiting the formation of ulcers in the (modified) Shay rat and in the Visscher starved rat. Compared on a molar equivalent basis, C-5473 was more active in the prevention of ulcers in the rat than either atropine or β-diethylaminoethyl xanthene-9-carboxylate methobromide. Likewise, in the Pavlov pouch dog, of all Trasentine analogues examined, C-5473 was the most active compound in suppressing the gastric secretory response to food. A comparison on a molar equivalent basis, both by the intravenous and oral routes, indicated that C-5473 was more active than β-diethylaminoethyl xanthene-9-carboxylate methobromide. C-5473 was very active in the Pavlov dog in doses which produced no significant side effects such as mydriasis, dysphagia or tachycardia; the latter obtained only with increments of dosage. Results with drug-induced gastric secretory studies in gastric fistula dogs will be presented. The relation between chemical structure and maximum activity in this series of compounds will be discussed.

Analgesic action of sodium gentisate. ROBERT C. BATTERMAN, LAWRENCE S. KRYLE* AND SIDNEY DANN.* *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.*

The effectiveness and safety of sodium gentisate were studied in 43 hospitalized and 54 ambulatory patients. The drug was administered orally as single or repeated doses of 0.3 or 0.6 gm. For hospitalized patients the period of observation ranged from one day to 55 days, during which time the effects of 1163 doses were followed. Control of pain regardless of severity, diagnosis or dosage administered was obtained in approximately 71% of the trials without the occurrence of a single instance of toxicity. Satisfactory analgesia occurred in 57% of the ambulatory patients who received the drug several times daily for several weeks. Untoward reactions were noted in 6 (11%) of the ambulatory patients and was within the range experience with placebos. The reactions were mild, insignificant and consisted mainly of transient nausea. Although the analgesic potency of sodium gentisate is equivalent to that of aspirin, the incidence of

effective analgesia for sodium gentisate is higher because of the little, if any, toxicity that may be encountered with its use.

Heat of the enzymatic inversion of sucrose. CARL R. BAUER AND MYRON J. LOVER (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

In a previous abstract (*Federation Proc.* 9: 287 1950) it was reported that thermistors may be used in microcalorimetry. This work has been continued and extended by using this method for determining the heat produced during the enzymatic inversion of sucrose. The changes in resistance of the thermistor were measured by a standard bridge circuit during a reaction run. The calibration of the calorimeter was obtained by passing a known amount of current through the heating element in the calorimeter. The calibration determinations were designed to reproduce very closely the same change in thermistor resistance as in the enzymatic experiments. Commercial invertase was used in these determinations which were carried out at 30°C. and a pH of 4.5. The reaction was stopped by the addition of 2 ml. of 1 N sodium hydroxide The initial concentration of sucrose was 0.1297 M. The amount of reducing sugar produced was determined by a gravimetric Fehling's analysis at the end of each determination. The results of 8 determinations gave the heat of enzymatic inversion of sucrose (ΔH) as -3.28 kg. cal. per mol. with an average deviation of ± 0.05 kg. cal. Comparable experiments made with an automatic recording microthermometer, actuated by a thermopile, gave an average value of ΔH to be -3.1 ± 0.2 kg. cal. per mol. for 6 determinations. In these experiments, the analyses were made polarimetrically.

Endocrine and autonomic systems in choline deficiency. JAMES H. BAXTER. *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Experiments were performed to determine if autonomic nervous system dysfunction, perhaps resulting in renal circulatory disturbances, might have been responsible for the renal lesions of choline deficiency in rats. The observation that subcutaneous injections of Dibenamine afforded protection, seemed compatible with this idea. However, severe local irritation together with adrenal cortical hypertrophy and thymic atrophy were noted with Dibenamine, and formaldehyde injections also appeared protective. An orally effective adrenergic blocking agent, and renal denervation, were without protective effect. Prostigmine likewise was ineffective, as was atropine. Observations of heart rate and other visceral functions afforded no clear evidence of autonomic dysfunction before development of the renal lesions. Because protection by Dibenamine appeared to be associated at least in part with 'alarming' effects of the injections rather than with specific blocking action, the effects of ACTH and cortisone were investigated. Both were found to afford significant protection against renal injury and death. Cortisone was more effective than ACTH, preventing the renal lesions in nearly half of the animals. A perhaps unconnected finding was that thiouracil prevented the lesions in nearly all animals. Morphological evidence that endocrine dysfunction might have preceded the renal lesions in deficient animals not subjected to other treatment was not at all conclusive, though changes characteristic of the 'alarm reaction' did occur spontaneously following development of the lesions. Paired-feeding techniques have not been employed, but effects of the substances and procedures on growth rate of the animals were taken into consideration in evaluating the results.

Action of bovine erythrocyte cholinesterase on neuromuscular transmission. I. T. BECK (introduced by K. I. MELVILLE). *Dept. of Pharmacology, McGill Univ., Montreal, Quebec, Canada.*

The action of Bovine Erythrocyte Cholinesterase (true Cholinesterase prepared and kindly supplied by Winthrop-Stearns Inc.) was investigated for its effect on neuromuscular transmission. The test preparations used were the rat.s gastrocnemius (*I*) and the cat's tibialis anterior (*II*) *in vivo* and the isolated rat's phrenic-diaphragm preparation (*III*). All were stimulated indirectly with supra- and sub-maximal single impulses twice or six times per minute. Up to 6000 U/kg. of the enzyme were injected intravenously in *I* and up to 2000 units (quick close intraarterially) in *II*. Up to 2000 U of Bovine Erythrocyte Cholinesterase were added to 50 ml. modified Tyrode solution bath in *III*. In all these cases the enzyme failed to alter the character of the normal twitches. Some possible explanation of the negative nature of these results will be discussed.

Effect of lethal doses of digitalis and digitoxin on tissue glutathione and attempts at BAL protection. JULIUS BELFORD AND GERTRUDE LANGE (introduced by CHANDLER MCC. BROOKS). *State Univ. Med. Center at New York City.*

To help ascertain the presence of a 'sulfhydril' mechanism in digitalis action, glutathione analyses of several tissues of digitalis and digitoxin treated cats were performed. Attempts were also made to protect animals against digitalis-digitoxin toxicity with BAL. Six control cats matched against 6 treated with tincture of digitalis (variation of U.S.P. XIII assay method) showed a statistically significant lowering of liver glutathione (247 mg. down to 118 mg.%; $P = .001$). Although lowering occurred in heart, kidney, and muscle,

this was not statistically significant. Pre-treatment with 10 mg/kg. BAL intramuscularly failed to protect; in fact toxicity was increased by 33%. In 12 control cats matched against 12 test animals, digitoxin also produced a statistically significant fall in liver glutathione (259 mg. down to 169 mg.%; $P = .01 - .001$). Again, though heart, kidney and muscle glutathione was lowered, there was no statistical significance. The blood level rose insignificantly. Pre-treatment with BAL (2 mg/kg. intramuscularly) produced a statistically doubtful protection of 5%. Plotting time of death (in all experiments combined) against liver glutathione content revealed a direct linear relationship—the earlier the death, the less glutathione.

Metabolism associated with positive inotropic action. DONALD BENNETT* AND MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

It has been previously reported that monofluoroacids exert a marked positive inotropic action on the hypodynamic papillary muscle in 10^{-2} M glucose-Ringers' solution and in substrate-free Ringers' solution. Sodium 4-fluorobutyrate is especially active and almost completely blocks the positive inotropic action of a succeeding dose of ouabain. Substrates have been altered to determine the mechanisms of action. Fluorobutyrate stimulates the papillary muscle similarly in glucose-free Ringers' solution, or when 10^{-2} M acetate, pyruvate, propionate, and butyrate are used as substrates. The addiction of 10^{-2} M acetate, pyruvate, propionate, butyrate, and valerate to a 10^{-2} M glucose-Ringers' solution abolishes the positive inotropic action of fluorobutyrate. The ability of glucose to block the positive inotropic action of fluorobutyrate in the presence of any one of the above metabolites is dependent upon concentration; 10^{-3} M glucose failing to block, whereas the block is obtained with 10^{-2} M glucose. Increasing the concentration of glucose to 10^{-1} M without the addition of another metabolite is sufficient to block the positive inotropic action of fluorobutyrate. If metabolic products of glucose oxidation such as 10^{-2} M lactate, or 10^{-2} M pyruvate are substituted for glucose, again in the presence of one of the above fatty acids in 10^{-2} M concentration, the positive inotropic action of fluorobutyrate is not blocked, but greatly enhanced. Galactose will partially replace glucose in these situations. These phenomena have been demonstrated on cat and monkey papillary muscles.

Effect of thiocyanate on blood electrolytes. ARTHUR BERNSTEIN, EMANUEL KLOSK, JAY BURGER, ALBERT M. SILVER AND FRANKLIN SIMON (introduced by HERBERT S. KUPPERMAN). *Newark Clin. Group, Newark, N. J.*

The modus operandi of the supposed therapeutic action of KCNS in hypertension has not been clarified although various hypotheses have been tendered in an attempt to explain the lowering of blood pressure by thiocyanates. The effect of thiocyanate upon serum sodium was studied in an attempt to elucidate the mechanism of action of the drug in the treatment of hypertension. Serum and urinary sodiums were determined in a series of ambulatory hypertensive patients before, during and after the administration of thiocyanate either alone or in combination with phenobarbital, sodium nitrite and rutin. The patients were maintained on a low salt diet for the duration of the study. Blood was drawn and 24-hour urine specimens were obtained at weekly intervals. There was a striking correlation between the blood sodium and blood thiocyanate level. When the blood thiocyanate approached a critical level there was a definitive drop in the serum sodium. The decrease in serum sodium was maintained as long as KCNS blood levels were elevated. Upon cessation of therapy and with a diminution of the blood KCNS to zero, there was an associated and concomitant rise in blood sodium. No significant changes in the serum sodium were noted during the control period when KCNS was eliminated from the regime and only phenobarbital, sodium nitrite and rutin were ingested. It is interesting to note that the prompt fall of blood sodium was noted long before the anticipated hypotensive effects of KCNS could occur.

Electrical effects produced by brain lipids in contact with neurohormones and drugs. REINHARD H. BEUTNER AND LOUISE WILLIAMS.* *Dept. of Pharmacology, Med. College of Alabama, and School of Dentistry, Univ. of Alabama, Birmingham.*

In previous studies of electromotive forces of oil cells (BARNES, BEUTNER *et al.*) the central conductor ('oil') between the aqueous solutions was invariably a non-biological substance such as a phenol derivative or nitro compound. We now have tested a native lipid extracted from the brain and kept anaerobically. The brain lipid was dissolved in an equal volume of chloroform. This solution was the central conductor, being in the cup of the oil cell submerged in saline, as usual. When tetraethylammonium chloride 1:1000 was added to the saline, the e.m.f. changed 45 mv., the negative pole being on the side where the drug was added. With addition of 1:100,000 or 1:10,000 of TEA the variation was insignificant. When TEA 1:100 was added, the negative variation was 80 mv. more negative than in TEA 1:10,000. A somewhat smaller change: 30 mv., in the same direction, occurred following addition of acetylcholine bromide or ephedrine sulfate 1:1000. Atropine produced larger variations viz: −95 mv. for a variation from 1:100,000 to

1:1000. *However when the brain lipid extract was exposed to air for 2 days, or longer, this electrical effect had disappeared.* This finding shows that the chloroform, used as solvent, is not responsible for the electrical effect, but only the brain lipid. The electrically active substance in it is obviously unstable. Atropine retained a slight electrical activity on the brain lipid even after exposure to air.

'Aramine': its enzymologic and sympathomimetic characteristics. KARL H. BEYER, HAROLD H. PECK,* CHARLES A. ROSS,* VIRGIL D. WIEBELHAUS* AND S. E. MCKINNEY.* *Dept. of Pharmacology, Med. Research Div., Sharp and Dohme, Inc., Glenolden, Pa.*

In large measure the oral efficacy, the duration of action and the renal elimination of a sympathomimetic amine are determined by the vulnerability of the agent to enzymatic inactivation in the body (BEYER: *Physiol. Rev.* 26: 169–197, 1946). These principles have been employed to anticipate the chemical structure of an amine that would combine the pharmacologic attributes of oral efficacy with a potency equivalent to an epinephrine ratio of about 1:18 and with a long duration of action. The structure of 'Aramine' (laevo-*m*-hydroxy-phenylisopropanolamine) confers on it the enzymologic and pharmacodynamic properties that have anticipated its suitability for oral, topical or parenteral administration, and its useful duration of vasoconstrictor efficacy.

Cross-transfusion in man: Effect on renal excretory products. HOWARD R. BIERMAN, RALPH L. BYRON, JR.,* KEITH H. KELLY,* GRACE SINGER* AND JOYCE SWADER.* *Lab. of Experimental Oncology, Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md. and Div. of Medicine, Univ. of California School of Medicine, San Francisco, California.*

Technics for continuous cross-transfusion have been developed in man, permitting the rapid exchange of hundreds of liters of blood in both patients. Cross circulation on 11 occasions has shown marked changes in the renal excretory products both in blood and in the urine. Excessive blood levels of urea and non-protein nitrogen can be lowered by cross-circulating with a patient with normal levels of these substances. During cross circulation between patients without azotemia there is a slight increase in non-protein nitrogen and urea which disappears within a few days. Cross circulation in man offers another technic in the study and possible treatment of anuria and related states of renal dysfunction.

Influence of respiratory movements upon the peripheral leukocyte level: control of the peripheral leukocyte level by the lungs. HOWARD R. BIERMAN, KEITH H. KELLY,* FAUNO CORDES,* NICHOLAS L. PETRAKIS,* MARILEE FOSTER* AND BETTY LOSE.* *Lab. of Experimental Oncology, Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md. and Div. of Medicine, Univ. of California School of Medicine, San Francisco.*

Previous work has revealed a mechanism within the lungs which is capable of removing billions of transfused leukocytes. Administration of histamine intravenously causes marked respiratory distress and is associated with a prompt decrease in circulating leukocytes. By catheterization of both right and left ventricles or aorta or large arteries in man this leukopenia has been shown to be caused by removal of these cells in the pulmonary circulation. The catheterization sampling technic during the Valsalva maneuver has shown a consistent and significant decrease in the peripheral leukocyte level due to removal of cells within the pulmonary circulation. With release of the valsalva there is a prompt increase of leukocytes from the lungs into the circulation well above the original level which may or may not persist. Other respiratory movements influence the circulating leukocytes which indicate an ebb and flow of leukocytes into and from the pulmonary circulation and determines, in part, the level of leukocyte number in the circulating blood.

Antispasmodic activity of a series of cinnamic acid esters and amides of diethylaminoethanol. MURRAY BLAIR,* ELLSWORTH B. COOK,* MICHAEL ROTHENBERG* AND BYRON B. CLARK. *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

A series of cinnamic acid esters and amides of diethylaminoethanol have been screened for antispasmodic activity on isolated rabbit ileum against acetylcholine as a spasmogen. The unsubstituted cinnamic acid ester of diethylaminoethanol is relatively inactive. Substitution of an α phenyl or an α phenoxy on the cinnamic acid increases the activity appreciably and to about the same degree. However, the β phenyl or β phenoxy substitution has no effect. The cinnamic acid amides are considerably less active than the corresponding esters. The substitution of dimethyl or dipropyl for the diethyl on the amino alcohol of the α phenoxy cinnamic acid amide of diethylaminoethanol does not change its activity appreciably. The general, quaternization of the amino group of either an acid ester or amide, as in α phenoxy cinnamic acid ester of diethyl methyl amino ethanol bromide, increases activity 2 to 3-fold. The three most active compounds are: α phenoxy cinnamic acid ester of diethyl methyl aminoethanol bromide, α phenoxycinnamic acid ester of diethylamino ethanol, α phenyl cinnamic acid ester of diethylaminoethanol.

Local emetic activity of digitalis glycosides. HERBERT L. BORISON, S. C. WANG AND T. D. HARRIS.* *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, and Dept. of Physiology, Columbia Univ., New York City.*

From studies on absorption of digitalis glycosides from the gut of cats, Gold *et al.* (*J. Pharmacol. & Exper. Therap.* 98: 337–345, 1950) concluded that vomiting after oral doses was due chiefly to a local gastrointestinal action. The discovery of a chemoreceptor trigger zone for emesis in the medulla oblongata by Wang and Borison (*Arch. Neurol. & Psychiat.* 63: 928–941, 1950) offers a new approach to the problem of differentiating between central and peripheral actions of emetic substances. Indeed, these workers have demonstrated this zone to be the site of central emetic action of certain cardiac glycosides (in press). In the present experiments, the trigger zone was ablated in dogs and cats. It was first established that these operated animals did not vomit following the intravenous injection of doses of digitalis glycosides effective in all normal animals tested. Scillaren A and lanatoside C were than administered orally and induced vomiting in the operated animals without any evident disturbance in threshold or latent period for emesis. Although these findings are ostensibly in agreement with those of Gold *et al.*, it is planned to proceed with visceral denervation procedures in both centrally operated and intact animals to provide a more critical analysis of the problem. Preliminary tests after transthoracic vagotomy alone did not indicate any significant change in response to scillaren A given orally.

Bile production by the isolated liver (motion picture). R. W. BRAUER, L. S. PEDIGO,* J. L. HILL* AND H. P. GLAVIANO.* *Depts. of Pharmacology and Experimental Therapeutics, and of Medical Illustration, School of Medicine, Louisiana State Univ., New Orleans.*

The method of preparation and operation of perfusion pump will be shown briefly. Several applications of the preparation (choleretics, dye excretion by the liver, effect of India ink, and of chloroform) will be shown. Bile flow, appearance of the organ, histological preparations, and graphic representation of results will be presented.

Application of surviving isolated rat liver to the study of bile production mechanism. R. W. BRAUER, R. L. PESSOTTI* AND M. P. FINLEY.* *Dept. of Pharmacology and Exptl. Therapeutics, School of Medicine, Louisiana State Univ., New Orleans.*

An isolated rat liver preparation which produces bile for as long as 30 hours has been previously described by the authors. The effect of anoxia, of temperature variations, and of variation of protein content of the perfusate on bile flow and bile composition will be discussed. A comparison of different choleretics has shown that the effects *in vitro* closely reproduce those seen in the intact animal with regard to bile concentration, but that the isolated liver is exceptionally sensitive to the effect of these agents. The action of various autonomic agents, including acetylcholine, epinephrine, nor-epinephrine, DFP and physostigmine, and tetraethylammonium ion have been explored. The application of these data to an understanding of the interrelation of structural and biochemical factors determining bile production will be discussed.

Cardiovascular actions of 2,3-benzodiazine-4-hydrazine (C-5968). J. BAYARD BRITTON,* JAMES P. TAYLOR* AND RAYMOND P. AHLQUIST. *Dept. of Pharmacology, Med. College of Georgia, Augusta.*

The effects of the thiazoline derivative, 2,3-benzodiazine-4-hydrazine, C-5968, on the cardiovascular system of the anesthetized dog have been studied. The most consistent effect produced by intravenous doses of 0.1–1 mg/kg. was a tachycardia apparently unrelated to any change in mean arterial pressure. The increase in heart rate occurred about one minute after the injection of the drug and persisted for 12–24 hours. Veratramine reduced the tachycardia, and the reflex slowing produced by the pressor agents such as ephedrine or epinephrine was unaffected. C-5968 produced a depressor response in about one-third of the animals receiving 2 mg/kg. About 2 minutes after administration the pressure declined over a period of 2–4 minutes to about 40–50 mm. Hg. This depressor effect was only temporarily antagonized by pressor agents. The pressor response to epinephrine was partially reversed with doses of 0.1–4 mg/kg., and the pressor response to arterenol was somewhat diminished. This partial adrenergic blockade was antagonized by ephedrine. The depressor effects of isopropyl arterenol and acetylcholine were potentiated by C-5968. None of the following were affected significantly by C-5968 in the doses tested: *1*) epinephrine myocardial stimulation, *2*) epinephrine induced cardiac arrythmia during chloroform anesthesia, *3*) cardiac inhibition of peripheral vagal stimulation, *4*) the pressor effect of central vagal stimulation, *5*) the carotid sinus reflex, *6*) the pressor effect of anoxia, 7) the depressor effect of intravenous histamine, and *8*) the pressor effect of vasopressin. Further studies on the cause of the tachycardia and on the reported renal dilation are being done. (Supported by a grant from the American Heart Association, Inc.)

Localization of Dibenamine in fat as a possible explanation of its duration of action. BERNARD B. BRODIE, LEWIS ARONOW,* ELWOOD TITUS* AND JULIUS AXELROD.* *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Dibenamine has been considered to exert its adrenergic blocking effect well beyond the period the drug remains in the body. An attempt was made to correlate the adrenergic blocking action of Dibenamine with plasma and tissue levels of the drug using a chemical method devised in this laboratory. It was found that after the intravenous administration of Dibenamine, concentrations in plasma and tissues declined within 2 hours to levels too low to measure (<0.5 mg/kg.) even though the adrenergic blockade persisted for several days. However, during the first 2 hours, concentrations in fat increased steadily to high levels and declined slowly over a period of several days. The prolonged duration of adrenergic blockade was found to be correlated with the concentration of Dibenamine in fat. This suggests that the compound in fat serves as a reservoir of drug which is slowly released to tissues. These results emphasize the importance of tissue localization in conditioning the activity of a drug. The metabolic fate of Dibenamine in the dog will be discussed.

Comparison of drug action in auricular flutter of aconitine and of circus movement origin. BARBARA B. BROWN (introduced by H. W. WERNER). *Pharmacology Dept., Wm. S. Merrell Company, Cincinnati, Ohio.*

Large dogs anesthetized with Dial were employed. Innervation to the heart was intact. Experiments were conducted in which the right auricle was isolated from the atrial region by an encircling crush. The subepicardial injection of aconitine (method of Scherf) on the body of the right auricle induced a rapid flutter confined to the auricle. Brief electric shocks applied near the sinus node induced a second and different flutter confined to the atrial and caval region. The effect of drugs on the second type indicated it to be a circus movement flutter. Procaine, DEAE (diethylaminoethanol), CDB (diethlaminoethyl 2, 4-dichlorobenzoate) and B-DCB y(diethylaminobutyl 2,4-dichlorobenzoate) injected intravenously affected the co-existing flutters differently. All four compounds slowed the aconitine flutter from rate of 600-300 down to 220 or to 0 for varying periods of time depending upon dose. Cooling of the site of aconitine injection slowed the flutter rate before drug injection but not afterward. The atrial flutter was generally markedly slowed for several minutes by procaine but the injection of B-DCB, DCB and DEAE usually caused it to revert suddenly to a sinus rate. The ventricular rate was increased by procaine and decreased by the other three substances. Similar results were obtained in experiments in which the auricle was intact and only one of the two types of flutter was induced, with the one exception that temporary reversions occurred occasionally following drug injection in the aconitine flutter.

Alteration of vasopressin induced blood pressure tachyphylaxis by antihistaminic drugs. JAMES S. BROWN,* ROBERT A. WOODBURY AND ROGER E. GIBSON.* *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

From male dogs anesthetized with sodium pentobarbital, 30 mg/kg., carotid blood pressure was recorded. Vasopressin (Pitressin) 0.3 U/kg. was administered at half-hour intervals until 3 or 4 injections demonstrated that the transient fall of blood pressure attributed to coronary constriction and cardiac weakness no longer occurred, and the pressor response was greatly reduced or abolished. Ten minutes prior to the next scheduled vasopressin injection, various antihistaminic drugs (equal molar basis) were given. Then vasopressin, in the same dosage, was observed to produce the transient fall in blood pressure, as well as the typical pressor response. Both of these effects had been increased by the intervening administration of certain antihistaminics but were usually less than the original control response. Myocardiographic tracings demonstrated that certain antihistaminics reestablished the transient cardiac weakness produced by vasopressin. The antihistaminics investigated were found to exhibit 'antitachyphylaxis' activity listed in the order of decreasing effect: Diphenhydramine (Benadryl), Tripelennamine (Pyribenzamine), Pyranisamine (Neoantergan), Thephorin, Doxylamine (Decapryn), Thenylene (Histadyl), Neohetramine, Chlortrimeton, Chlorcyclizine (Perazil). Various other drugs have been utilized such as procaine, sympathomimetics, adrenergic blocking agents, histamine and acetylcholine infusions, and atropine in an attempt to reveal the mechanism(s) through which the antihistaminics altered tachyphylaxis and restored partial responsiveness to vasopressin. (Supported by a U. S. Public Health Research Grant and by the American Heart Association.)

Pressor dose-effect curves for l-epinephrine, l-nor-epinephrine and a commercial epinephrine. ROBERT V. BROWN AND GALE C. BOXILL.* *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

For each of the two pure compounds 10 dogs were given progressive logarithmically spaced doses. Twenty dogs were given the commercial compound. All were anesthetized with 30 mg/kg. of pentobarbital sodium intravenously, and fully

atropinized by the U. S. P. XIII method. The dose-effect curves are all rectangular hyperbolas having the equation: $mxy + ny - x = 0$, where x is in μg. of base per kg. of body weight, y is the rise in blood pressure in mm. Hg, and m and n constants so chosen that $1/m$ is the asymptote parallel to the x axis and $-n/m$ the asymptote parallel to the y axis. For 1-epinephrine, n = .03264, m = .00812; for the commercial epinephrine (presumably containing 1-norepinephrine) n = .01218, m = .00834; for 1-nor epinephrine n = .00578, m = .00915. The squares of the semitransverse axes of these hyperbolas are respectively 495.3, 175.1, and 69.1. Potency ratios are the reciprocals of the ratios of these last figures; hence, the potencies, referred to 1-epinephrine as unity, are 1.0, 2.83, and 7.18, respectively. The values of m and n were determined by locating the center of each hyperbola by the method of least squares, using the Legrange method of the undetermined multiplier. There is no evidence of any significant vasodilating action of any of these compounds until a dosage level of about 5 μg/kg. is reached; there is then a marked after-depression of blood pressure.

Reaction of man to intravenous gallium citrate. H. D. Bruner. *Med. Div., Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.*

Patients with far advanced osteogenic sarcomata are being treated with radioactive Ga^{72} which segregates in bone. At present, comparatively large amounts of stable carrier gallium must be injected and the radiation therapy is limited by the patients' reactions which are interpreted as a form of heavy metal poisoning. Gallium as the citrate complex at pH 7.2–7.4 has been administered by slow i.v. drip mixed with saline. Patients have received as many as 9 injections spaced twice a week in doses varying between 1.0–7.5 mg. of Ga metal ion/kg., with an average of nearly 3 mg/kg.; the largest total dose was 41.6 mg. Ga/kg. and 11.2 μc/kg. over 42 days. Transient nausea and a bad taste in the mouth was associated with the injection itself; as treatment continued nausea, vomiting, and anorexia became pronounced, yet weight loss was slight or absent. The white cell count and hemoglobin values began to decrease after the 3rd to the 5th dose, necessitating eventually cessation of therapy and transfusions. The appearance of albumin in the urine of some suggests a moderate nephrotoxic action of Ga. In 3 patients who received larger cumulative doses the skin reacted with spider angiomata, a maculo-papular rash, or a dry grey brittle appearance. Except for active dislike for the injection itself, the patients were depressed and somewhat apathetic. Excretion studies definitely show accumulation of Ga within the body yet clinical recovery promptly followed cessation of treatment. Most of these reactions are considered the result of the metal rather than the radiation.

Anesthesia. XXXV. Local anesthetic action of metahydroxyprocaine. Raymond M. Burgison,* John C. Krantz, Jr., C. Jelleff Carr, James F. Vitcha* and Ruth D. Musser.* *Dept. of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.*

Many compounds having analogous structures have been introduced into the practice of anesthesiology since the synthesis of procaine in 1905. Some of these substances have exhibited certain advantages over the action of procaine, but in most instances potency as a local anesthetic and systemic toxicity have paralleled each other as properties of these agents. Procaine, therefore, has remained the most generally-used of all the local anesthetics. Metahydroxyprocaine (diethylaminoethyl - 3- hydroxy - 4- amino benzoate) has been synthesized and studied as a local anesthetic. When used in a 2% aqueous solution as the sodium or potassium salt, metahydroxy exhibited greater anesthetic potency than procaine when tested on the frog's foot (Turch method), in the rabbit's cornea, and by the guinea pig sciatic nerve method (Schackell), but when compared to procaine hydrochloride by the dog sciatic nerve method under faradic stimulus, by the human intradermal wheal method, and as a spinal anesthetic in dogs, the compound appeared to possess no greater potency than procaine. Toxicity studies on metahydroxyprocaine revealed that this compound is less toxic than procaine hydrochloride. The MLD averaged 72 mg/kg. versus 44 mg/kg. by intravenous injection into the rabbit; the LD_{50} by intraperitoneal injection into the rat was found to be 240 mg/kg. versus 184 mg/kg.; and in the mouse, 220 mg/kg. versus 180 mg/kg. Electrocardiograms observed on dogs during and after injection of 1 cc. of 2% sodium metahydroxyprocaine aqueous solution were essentially normal.

Sympathetic ganglionic blocking activity of trasentine hydrochloride and some of its homologues. Anne Cameron,* Frederick F. Yonkman and Albert J. Plummer. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Trasentine hydrochloride and some of its homologues (R. Meier and K. Hoffmann, Helv. Med. Acta., 7: 106, 1940/41) have been studied with respect to their ability to block transmission of electrical impulses through the superior cervical sympathetic ganglion after perfusion of the latter through its arterial supply. The criterion used was the response of the feline nictitating membrane to faradization of preganglionic cervical

sympathetic fibers. The number following the name of each compound studied indicates the relative activity of the compound in comparison with Trasentine, assigned the value 1.0: C-2673/2, diethylaminoethyl diphenylacetate methyl bromide, (1.0); Trasentine 6H, (1.6); C-3854/1, diethylaminoethyl phenylcyclohexylacetate methyl bromide, (5.0); C-3762/4, diethylaminoethyl dicyclohexylacetate methyl bromide, (2.5); C-5473, diethylaminoethyl phenylcyclohexyl hydroxyacetate methyl bromide, (5.0); C-6642, dimethylaminoethyl diphenylacetate hydrochloride, (1.0); C-6643, dimethylaminoethyl diphenylacetate methyl bromide, (0.5); C-6647, dimethylaminoethyl phenylcyclohexylacetate methyl bromide, (5.0). The values are approximate, since differences in duration of action have not been considered. By the use of nicotine, one can distinguish between drugs possessing only a local anesthetic action and drugs possessing only specific ganglionic blocking action, since nicotine will stimulate the ganglion after the former but not after the latter.

A correlation of responses of the cardiovascular system of man with different methods of anesthetic administration. ROBERT T. CAPPS*, GORDON GARNETT* AND O. SIDNEY ORTH. *Depts. of Pharmacology and Anesthesiology, Univ. of Wisconsin Med. School, Madison.*

Different methods of administration of general anesthetic agents, open, semi-open, semi-closed or closed, cause variations in pressures in the respiratory tree, altered efficiency in heat loss from the body, and variations in composition of the respired atmosphere—particularly in its oxygen and carbon dioxide content. Independently or collectively such changes may cause altered cardiovascular efficiency from resulting cardiac arrhythmia, periods of hypertension or hypotension, changed cardiac output, and other physiological upsets. In the present study, after preoperative evaluation of patients, the determination of degree of blood oxygen saturation, percentage of oxygen and carbon dioxide in the respired atmosphere, and cardiac rhythm was made during operation. A close post-operative follow-up was made until the patient's discharge from the hospital. Changes for those individuals with normal versus those with diseased cardiovascular systems, as influenced by variations of the factors due to the method of anesthetic administration, are being compared to determine if a particular method may be more advantageous in specific types of heart disease.

Effect of nitrites and nitrates on arterial adenosine triphosphatase. C. JELLEFF CARR, JOHN C. KRANTZ, JR. AND HAROLD C. BRYANT.* *Dept. of Pharmacology, Univ. of Maryland, Baltimore.*

The vasodilation produced by nitrites and organic nitrates has been studied to locate in arterial tissue the target enzyme system upon which these vasodilators act. The contractile mechanism which specializes skeletal muscle for quick movement is presumably the same as that which enables smooth muscle to maintain its tonus. Evidence suggests that ATP plays an important role in the maintenance of smooth muscle tone and contraction. Therefore the influence of nitrites and organic nitrates upon the ATP-ATPase system of the aorta of the rabbit was studied. ATPase activity is inhibited by the rapidly-acting vasodilators such as amyl nitrite, glyceryl trinitrate and isobutyl glycollate nitrate. This means the failure of ATP to break down under the influence of these drugs. If through the decomposition of ATP by ATPase, tone and constriction of the artery are maintained, it is possible that a drug which acutely relaxes the artery may do so by interfering with the activity of ATPase. Thus the vessel would respond by relaxing. The ATPase activity is not inhibited by sodium nitrite. It is a slower-acting vasodilator and it is possible that it affects the energy system of the contractile mechanism at another segment, viz., oxygen uptake or glycolysis. The ATPase of artery is particularly vulnerable to these drugs for ATPase from uterine smooth muscle and skeletal muscle is unaffected by the presence of these vasodilators.

Influence of size of infarct, lanatoside C and quinidine on incidence of ventricular fibrillation after coronary occlusion. ROBERT CASE, GEORGE MISRAHY, LOOMIS BELL, ROBERT NIMS AND WILLIAM PYLES (introduced by A. GILMAN). *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and St. Luke's Hosp., New York City.*

Dogs were anesthetized with sodium pentobarbital and, under artificial respiration, the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was dissected. Arterial blood pressure and electrocardiogram were recorded. The dogs were observed for 4 hours after the ligation of the coronary artery. *I*) A 'large' infarct was produced in 15 dogs by ligating the left anterior descending coronary artery just below its junction with the circumflex branch. Ventricular fibrillation occurred in 73% of the 15 dogs. In a series of 10 dogs in which a 'small' infarct, estimated to be ¼–⅕ the size of the 'large' infarct, was produced by tying the coronary artery much lower along its course, fibrillation occurred in 10% of the dogs. *II*) In a series of 10 dogs, a 'small' infarct was produced and 0.06 mg. of lanatoside C per kg. of weight was injected intravenously within 5–12 minutes, at the time of the arterial ligation. Fibrillation did not occur in any

of the 10 dogs as compared with an incidence of 10% in the control series. *III*) A 'large' infarct was produced in 15 dogs in which at the time of the arterial ligation the plasma quinidine level ranged between 0.72 and 11.3 mg. per liter. Fibrillation occurred in 60% of the dogs as compared with an incidence of 73% in the control series.

Drugs causing the Bezold-Jarisch reflexes (Veratridine, potassium, adrenaline and acetone); their carotid and intracranial actions. A. Cerletti,* T. H. Li,* J. Alanis* and D. M. Aviado, Jr.. *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*

Several drugs including acetone cause Bezold-Jarisch reflexes. Because of striking similarity between reflex drug and pressoreceptor activities in the heart and lungs (*Federation Proc.* 9: 255, 1950), it has been repeatedly suggested that drugs stimulate presso-receptors to cause the Bezold-Jarisch reflexes. The dog's carotid sinus (morphine and chloralose anesthesia) is utilized to investigate pressoreceptor sensitivity to drugs. An intracarotid injection of veratridine (2 μg.) causes hyperpnea vasodilatation and bradycardia. After cutting the sinus pressor fibers, respiratory and circulatory stimulation result which (like cyanide effects) are eliminated by cutting sinus nerve. The drug reaches the pressoreceptors not through the lumen of the internal carotid or its sinus but through the external carotid. Adrenaline causes similar effects before the onset of its well-known systemic action. After complete carotid denervation, intracarotid or occipital arterial injections of veratridine and potassium cause depression of respiration and circulation. If the same or larger dose is injected into the vertebral artery, stimulation of respiration and circulation is the most usual result. The two forms of intracranial responses probably means that these drugs besides stimulating medullary centers (vertebral artery) also stimulate (occipital artery) either an inhibitory sensory tract or a group of intracranial receptors so far unknown.

Inability of Coprini to sensitize man to ethyl alcohol. George P. Child. *Dept. of Physiology and Pharmacology, Albany Med. College, Albany, N. Y.*

A number of references have appeared in the literature which have ascribed to Coprinus atramentarius and C. comatus the property of sensitizing man to alcohol. It is alleged that if a small amount of alcohol is imbibed after eating these fungi, man becomes violently intoxicated, disoriented and irrational for periods from 6–24 hours. In a series of separate tests, C. atramentarius, comatus and micaceus were consumed raw, parboiled or fried lightly in butter in amounts ranging from 100–600 grams. During and after these feedings the subject consumed an alcoholic beverage in an amount sufficient to indicate whether alcohol sensitization had occurred. No sensitization to ethyl alcohol was found. It was noted during the collection of the fungi that Paneoli often grew in the vicinity of the Coprini and could easily be mistaken for the latter. Accordingly, a small amount of Paneolus was consumed, without alcohol. The subject's face became flushed, his eyes were glassy, the pulse rate increased and a slight dyspnea was experienced. It is suggested that reports which allege that Coprini sensitize to alcohol might be explained by the accidental inclusion of Paneolus in the cooking vessel.

Inability of cobalt to produce polycythemia in rats treated with ethylenediamine tetracetic acid. George P. Child and Peter Leonard.* *Dept. of Physiology and Pharmacology, Albany Med. College, Albany, N. Y.*

Ethylenediamine tetracetic acid (EDTA) reacts with most metallic ions to form soluble nonionic metal chelate compounds. The stability of these complexes is so great that the metallic ions are practically completely inactivated. It was of interest to determine whether this property of EDTA would apply to a number of biological systems. It was found in rats that the toxicity of a number of heavy metals such as mercury, copper, and lead could be reduced by the sodium salt of EDTA. In more extensive experiments the inhibition of the polycythemia inducing property of cobalt was used as a measure of EDTA reactivity. Thirty female rats, each weighing about 200 gm., were divided into six groups. *Groups 1–4* were given food containing 0.1% cobalt acetate. *Groups 2, 3,* and *4* were given food containing 0.2%, 1.0%, and 5.0% EDTA in addition to the cobalt. *Group 5* received 5.0% EDTA without cobalt and *group 6* received neither EDTA nor cobalt. After four weeks the rats were sacrificed and their hemograms were determined. *Group 1* had the usual cobalt *groups 2* and *3* displayed some inhibition of this effect. There were no essential differences among the blood counts of *groups 4, 5,* and *6*. These results indicated that 5% EDTA in the food of rats completely inhibited the polycythemia inducing property of 0.1% cobalt acetate.

Vasodepressor activity of adenosine, 2-chloroadenosine and related nucleosides. D. A. Clarke,* J. Davoll,* F. S. Philips and G. B. Brown. *Pharmacology Section, Div. of Exper. Chemotherapy, and Div. of Protein Chemistry, Sloan-Kettering Inst. for Cancer Research, New York City.*

Certain nucleosides and purine bases have been

compared with adenosine for relative vasodepressor activity in cats. Intravenous injection of adenosine results in precipitous vasodepression followed by prompt recovery which may be related to enzymatic deamination of adenosine. The probable importance of deaminases in limiting duration of adenosine action prompted development of manometric methods to study deamination of nucleosides; erythrocytes are a source of deaminases. 2-Chloro-adenosine is a potent vasodepressor; 5 μg/kg. causes vasodepression approximately equivalent to 2 mg/kg. of adenosine. However, recovery following 2-chloro-adenosine is significantly delayed. Larger doses cause shock-like reactions characterized by low blood pressure, respiratory failure and death. 2-Chloro-adenosine is not deaminated by adenosine deaminase; it inhibits deamination of adenosine but not sufficiently to account for the marked vasodepression. The necessity of 6-amino groups for vasodepression characteristic of 9-β-D-ribofuranosylpurines has been established. 2-Position substitution in adenosine by hydroxy-, acetamido- or chloro- exaggerates depressor activity. 2-Amino substitution does not increase vasodepression. 6-Amino replacement by hydroxyl forms compounds (inosine, guanosine) without appreciable depressor activity. These observations emphasize the specific configuration related to vasodepression by nucleosidic purine moieties, particularly the importance of 2- and 6-positions. Adenosine derivatives bearing 2-position substituents may not only resist adenosine deaminase action but also may exert greater, more prolonged vasodepression than adenosine.

Effects of estrogens and progesterone on motility of the feline uterus in vivo. MARY LEE CLARY,* ANNE CAMERON* AND BRADFORD N. CRAVER. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Experiments involving injections of either or both progesterone and an estrogen at given intervals before and during the testing of synthetic uterotonics have been performed. Good uterine responses to oxytocics were inconsistently elicited from cats in natural proestrus or estrus. The uteri from cats in other stages of the estrous cycle were entirely unresponsive to both Pitocin and synthetic oxytocics. Either estrogenic or gestational hormones given alone at variable intervals prior to the day of the experiment induced some responsiveness of the uterus to oxytocics, but only after both an estrogen and progesterone had been injected (Clauberg's procedure with and without modifications) could a maximal responsiveness of the uterus be assured. In occasional normal cats an estrogen alone gave maximal but temporary responsiveness. The uteri of immature and ovariectomized cats were entirely unresponsive to oxytocics and if treated with estrogen alone were but poorly and briefly responsive. The uteri of ovariectomized cats treated with progesterone alone exhibited a slight response to drugs. These results would seem to support the concept that there is a synergistic action between progesterone and estrogens, rather than an antagonistic action in respect to uterine motility. Despite the injection of a wide variety of hormones and drugs during an experiment in which oxytocics were being tested, it did not prove possible to restore the responsiveness of an inert uterus to drugs.

Inhibitory effects on the feline uterus in vivo of epinephrine, nor-epinephrine and N-isopropyl nor-epinephrine. MARY LEE CLARY* AND BRADFORD N. CRAVER. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

In over 50 experiments it has been demonstrated that progesterone (in the presence of an estrogen) enhances the inhibitory effects of nor-epinephrine on the cat uterus *in vivo* but not the inhibitory effects of epinephrine and N-isopropyl nor-epinephrine. To produce the same degree of relaxation by nor-epinephrine in the estrogen-primed cats as in the cats primed with both an estrogen and progesterone it was necessary to administer an adrenolytic to minimize the pressor responses. Priscoline proved most satisfactory for this purpose because it did not alter the control level of blood pressure. In ovariectomized cats treated only with an estrogen, nor-epinephrine moderately stimulated the uterus, although epinephrine was still inhibitory in its action. In ovariectomized cats treated only with progesterone, both epinephrine and nor-epinephrine increased the tonal level of the uterus; only N-isopropyl nor-epinephrine remained inhibitory in its action. The cause of these findings would appear unknown but the results may offer clues to two unsolved problems associated with the uterus; *1*) what initiates the onset of labor; *2*) why should epinephrine exert opposed effects upon the uteri of some species dependent upon whether pregnant.

Toxicity and anticholinesterase action of an alkyl coumarin thiophosphate (E838). KENNETH W. COCHRAN,* JOHN DOULL* AND KENNETH P. DUBOIS. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

The diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin (E838) is chemically related to parathion and exhibits similar insecticidal properties. The present investigation was conducted to measure the toxicity of this compound and to investigate its pharmacological actions. For toxicity tests solutions of E838 (Geary Chem-

ical Corp.) in 10% ethanol-90% anhydrous propylene glycol were used. The approximate LD_{50} for rats was 15 mg/kg. intraperitoneally and 22 mg/kg. orally. No significant age or sex differences in susceptibility were observed. Mice and guinea pigs resembled rats in susceptibility. Rats tolerated daily injections of 2 mg/kg. for 20 days but daily doses of 3 mg/kg. resulted in 17% mortality and 5 mg/kg. daily produced 50% mortality in 20 days. Within 15 minutes after parenteral administration of acutely toxic doses symptoms of generalized parasympathetic and central stimulation occurred. A final concentration of 5×10^{-9} M E838 produced 50% inhibition of rat brain cholinesterase *in vitro*. In 15 minutes after 9.4 mg/kg. intraperitoneally the cholinesterase activity of brain, serum, skeletal muscle, submaxillary gland, and ileum was inhibited 69–85%. This level of inhibition persisted for about 2 hours and the enzyme activity then gradually returned to normal within the next 3 days. These experiments demonstrated that E838 is a potent inhibitor of cholinesterase *in vitro* and *in vivo* producing nicotinic and muscarinic effects which are reversible after sublethal doses.

Needless use of a narcotic in preanesthetic medication. ELLIS N. COHEN* AND HENRY K. BEECHER. *Anesthesia Lab. of Harvard Med. School at Massachusetts General Hospital, Boston.*

The role of a narcotic as a premedicating agent for anesthesia is currently being evaluated by a controlled clinical and laboratory study. Notwithstanding the long tradition of use of a narcotic here its use is questioned. Three premedicating solutions containing atropine and morphine, pentobarbital sodium and atropine, or atropine alone are administered as unknown solutions on a patient-weight basis. Neutral questionnaires evaluate the patient's mental status preoperatively while under the influence of the premedicating drugs, the anesthetists objective observance of the patient's status, and finally the patient's postoperative recall of his anesthetic emotional experience. The anesthetist further records an opinion as to the character of the anesthetic induction, the adequacy of the premedication, and whether he thinks a narcotic drug was used. Venous blood levels of anesthetic gases, drawn during surgery provide a base line to calculate any contribution of the premedicating drugs to the surgical anesthesia. The total dose of thiopental sodium used in comparable operations is compared with and without a narcotic in the preanesthetic medication. Neither the patient nor the anesthetist can detect the presence of morphine (15 mg. dose) in the preanesthetic medication, nor does the morphine influence the blood levels of ether or cyclopropane required. No clear reduction in thiopental sodium is effected by the morphine.

Ganglion blocking activity of aliphatic quaternary amines and related alkyl piperidinium salts. DONALD L. COOK AND MARTIN M. WINBURY (introduced by W. E. HAMBOURGER). *Div. of Biological Research, G. D. Searle and Company, Chicago, Ill.*

Studies on a group of 20 ganglion blocking compounds have shown that piperidines can be considered as ring-closed aliphatic quaternary amines. On a molar basis the piperidines are quantitatively similar in ganglion blocking activity to the aliphatics. Certain basic structure-activity relations can be demonstrated with these two groups. Ethyl substituents on the quaternary nitrogen are more effective than methyl substituents. Hydroxyethyl is less active than ethyl. Substitution of methyl on the carbons adjacent to the quaternary nitrogen increases ganglion blocking potency. As a consequence the most active member of each series is SC-1950, 2,6-dimethyl-1, 1-diethyl piperidinium bromide (Cook *et al. J. Pharmacol. & Exper. Therap.* 99: 435, 1950) and methyl, ethyl, diisopropyl ammonium bromide. Experiments indicate that the aliphatic quaternary amines and the related alkyl piperidinium salts are 'pure' ganglion blocking agents and are devoid of peripheral autonomic activity.

Evaluation of the isolated rabbit ileum as a quantitative determination of antispasmodic activity. E. B. COOK,* MURRAY R. BLAIR* AND BYRON B. CLARK. *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

Ten controlled experiments were performed on isolated rabbit ileum to compare the antispasmodic activity of a known (atropine) against an unknown compound (an acid ester of diethylaminoethanol) using acetylcholine as spasmogen. Each experiment consisted of matching tissue responses to a high and a low concentration of the known and unknown randomized by a latin square 4×4 design (*J. Pharmacol & Exper. Therap.* 92: 260, 1948). Individual experiments permitted the utilization of the known and unknown drugs on tissues from the same animal, allowing an evaluation of a 2-point versus a 4-point assay. Pooled estimates from 5 experiments, in which 4 different concentrations of known and unknown were the same, were plotted on logarithmic-probit paper and analyzed for parallelism, which was substantiated. The calculated regression lines for the 4-point analysis were tested by Chi square yielding P values of 0.29 and > 0.001 for the unknown and known, respectively. The variability in the response to atropine probably accounts for the significant deviations, but the evidence is insufficient to reject the linearity hypothesis. The same general relationships hold in the 2-point analysis. Because the atropine variability, and the population studied (N = 40) are too large for routine

quantitative evaluations of antispasmodic activity, studies are in progress to develop a simpler and more reliable technique. The differential sensitivity of the tissues to atropine (0.00075 mg%–0.0015 mg%) as compared to the unknown (0.04–0.1 mg%) indicates the need for further study.

Action of common analgetic materials on spinal reflexes. LEONARD COOK* AND D. D. BONNYCASTLE. *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*

It was reported by Houde that the spinal rat can react to a noxious stimulus, and that this reflex movement can be depressed with analgetic materials. This finding has been confirmed with a modified D'Armour-Smith apparatus by examining representative samples of weak, moderate and strong analgetic materials in both acute and chronic spinal rats. Methadone (1.5–6.0 mg/kg.), morphine (1.5–6.0 mg/kg.) and codeine (5–20 mg/kg.) produced a significant elevation of stimulus-threshold in both acute and chronic spinal rats. Isonipecaine has been found to have only slight depressant effects in the same dosage range upon the spinal preparation as compared with the intact rat. In view of these findings the spinal reflexes and ganglionic transmission were studied in an attempt to elucidate possible sites of action of these drugs. Internuncial neuron studies were carried out in cats, involving knee jerk, flexor reflexes, crossed extensor reflexes and other spinal reflexes. Ganglionic transmission was also studied in cats, involving the superior cervical ganglion. The effect of morphine, methadon, heptazone, dilaudid, isonipecaine, hycodan, codeine, Nu-1779, Nu-1196, aminopyrine, antipyrine, and aspirin upon spinal cord activity as estimated by the above procedures will be reported. It is to be noted that in all such experiments the dosage on a mg/kg. basis extended from below the recommended therapeutic dose to the near-toxic level.

Adrenal steroid excretion in nitrogen trichloride ('agene') epilepsy. P. J. COSTA* AND D. D. BONNYCASTLE. *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*

In a previous communication it was reported that a decrease in urinary neutral 17-ketosteroid excretion occurred in dogs fed convulsive levels of 'agenized' material. This finding suggested that stress of this type altered adreno-cortical metabolism. These studies have been extended to include, in addition to 17-ketosteroid excretion, the corticoid excretion and eosinophile levels as indices of adrenal activity. Steroid excretion patterns have been followed in a number of types of stress including convulsions produced by 'agene' and metrazol, surgical procedures and anesthesia. It would seem from these experiments that under the different conditions of stress there are alterations in the various adrenal cortical secretions. 'Agene' convulsions produce a decrease in 17-ketosteroid excretion and an increase in corticoid excretion, while on the other hand, metrazol-induced convulsions result in an increase in both 17-ketosteroid and corticoid excretion. The effects of various, hormones (ACTH, Cpd. E, DCA, testosterone, progesterone) in modifying these seizures will be considered.

Effectiveness of Dibenamine in blocking inotropic action of epinephrine and corresponding ineffectiveness with Digitalis. M. DEV. COTTEN* AND R. P. WALTON. *Dept. of Pharmacology, Med. College of South Carolina, Charleston.*

The typical marked increments in heart contractile force produced by epinephrine are largely abolished following high doses of Dibenamine. The conditions of these experiments with open-chest vagotomized dogs have been previously described (*J. Pharmacol. & Exper. Therap.* 98: 346, 1950; *Am. J. Physical* 161: 489, 1950). Dibenamine (20 and 40 mg/kg. by i.v. infusion), after acting for 45–90 minutes, reduced the contractile force increments produced by test doses of epinephrine (3 γ/kg.) from about 80% to about 15%. Pressor effects were much more readily blocked than the inotropic and chronotropic effects. Under comparable conditions of Dibenamine action, the positive inotropic effects of digitalis (0.75 CU/kg. by i.v. infusion) were not significantly altered. Digitalis was not administered until test doses of epinephrine demonstrated that typical inotropic responses to epinephrine had been reduced to about 25% of that in the period preceding Dibenamine. Under these conditions, the responses to digitalis consisted in an average increase in contractile force of about 70%; this is approximately the grade of response observed in a previous study of cardiac glycosides. Accordingly, Dibenamine is considered to be capable of differentiating the positive inotropic action produced by epinephrine as contrasted with that produced by digitalis. Further experiments with erythrophleum alkaloids (0.5 CU/kg. by i.v. infusion) showed that they behaved like digitalis. Veratrine alkaloids, on the other hand, behaved closely like epinephrine in that the inotropic action was largely abolished by Dibenamine. Following Dibenamine, ECG effects with epinephrine were approximately diminished to the same degree as the inotropic action; ECG effects with digitalis, on the other hand, were typically manifested after Dibenamine.

Acute toxicity of salicylic, p-aminobenzoic and p-aminosalicylic acid, alone and in combination. GEORGE CRONHEIM (introduced by GEORGE MAISON). *Research Lab., S. E. Massengill Company, Bristol, Tenn.*

In recent years, the group of clinically useful substituted benzoic acids has been enlarged by *p*-aminobenzoic acid (PABA) and *p*-aminosalicylic

acid (PAS). Since the therapeutic doses are large and since these drugs are also given instead of or together with salicylic acid (SAL), their relative toxicity alone and in combination is of interest and has been determined. Acute oral LD_{50} (24 hours) in gm/kg. for rabbits with 95% confidence limits: SAL—1.35 (1.20–1.51); PABA—1.83 (1.73–1.94); PAS—3.65 (3.35–3.98). In mixtures of equal parts: SAL plus PABA—1.35 each (1.27–1.43); SAL plus PAS—0.96 each (0.83–1.11). Subacute oral LD_{50} in gm/kg. (9 daily doses) for rats: SAL—0.55 (0.49–0.61); in mixtures with SAL kept constant: SAL 0.50 plus PABA 0.75 (0.50–1.08); SAL 0.50 plus PAS 0.80 (0.59–1.05). Lethalities did not occur after doses of 2 gm/kg. of either PABA or PAS. Blood SAL determinations in rabbits were not altered by simultaneous administration of either PABA or PAS. In contrast PAS blood levels were altered by SAL in a manner indicative of a reduction in the rate (but not in the total amount) of absorption from the gastrointestinal tract. It was concluded: SAL was more toxic than either PABA or PAS in both acute and subacute tests. The acute toxicity of SAL for rabbits was enhanced by equal doses of PAS. The subacute toxicity of SAL for rats was not intensified by either PABA or PAS when the latter was present up to 1.5 times the amount of SAL.

Anesthetic properties of xenon in animals and human beings with some additional observations on krypton. STUART C. CULLEN AND ERWIN G. GROSS. *Div. of Anesthesiology, Dept. of Surgery and Dept. of Pharmacology, State Univ. of Iowa College of Medicine, Iowa City.*

When mixtures of 80% krypton and 20% oxygen were used, either with or without premedication, no unequivocal signs of narcosis could be demonstrated in animals. Inhalations by three human subjects of krypton produced only slight dizziness and discomfort, and it was concluded that krypton had no significant narcotic properties. Similar animal experiments were repeated using xenon and comparisons with nitrous oxide were made. Some evidence of narcosis was obtained, especially in the premedicated rabbits and there was pronounced diminution in response to painful stimuli. Six human subjects inhaled either a 50-50 mixture of xenon and oxygen or a 50-50 mixture of nitrous oxide and oxygen from a closed system spirometer with soda lime in the circuit. Oxygen was added according to the metabolic requirements of the individual. Pain threshold measurements by the Hardy-Wolff-Goodell apparatus indicated a uniform 15% increase in pain threshold with either gas mixture. Each subject reported more pronounced subjective symptoms of narcosis with the xenon mixture than with the nitrous oxide. Two human subjects inhaled mixtures containing 70% of xenon, 30% of oxygen for 3 minutes and found pronounced narcotic effects with partial loss of consciousness. In both patients, loss of consciousness occurred in less than 5 minutes and recovery in about 2 minutes. It appeared that xenon had anesthetic properties approximately equivalent to ethylene.

Sex differences in response of the rat to ACTH. JACK M. CURTIS, ERNEST J. UMBERGER AND MARY H. MCGOVERN (introduced by R. L. GRANT). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Sex differences in the size of the adrenals of the rat are commonly observed. Further, the influence of either estrogen or androgen treatment on adrenal size is not the same in the two sexes (Review by TEPPERMAN, ENGLE AND LONG. *Endocrinology* 32: 373, 1943). In studying the ascorbic acid depletion methods for the assay of ACTH, differences in response which appeared to be due to the sex of the assay animal were observed. In order to evaluate these differences, the responses to 3 dosage levels of ACTH (Armour's LA1A) were determined in the following groups of animals: *group I*, hypophysectomized male rats; *group II*, hypophysectomized castrate male rats; *group III*, hypophysectomized female rats; and, *group IV*, hypophysectomized castrate female rats. The responses to each dosage level and the slope of the regression curve for *group I* rats were greater than for any other group. *Groups III* and *IV* were very similar and *group II* resembled *groups III* and *IV* rather than *group I*. Morphological changes, either atrophy or hypertrophy, in the adrenal glands are often interpreted to indicate changes in the functional activity of the glands. If the results of the ascorbic acid depletion method are a measure of the functional activity of the adrenals, the data presented indicate directly a sex difference in adrenal activity. The hypophysectomized male rat is the animal of choice for use in the assay of ACTH.

Mechanism of action of synthetic tetrahydrocannabinol (Parahexyl). MOHAMED ALI YOUSEF DAHI (introduced by E. M. K. GEILING). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Parahexyl as a solution of 80% in propyleneglycol, when injected intraperitoneally in doses of 1 gm/kg. in rats and guinea pigs, causes a marked rise in the blood sugar level in from 5–10 hours. It was also shown that the drug exerts a protective action on the liver glycogen of the normal animals. In studying the glycogen synthesis from glucose and the phosphorylated intermediates in the starving and glucose fed guinea pigs, the drug was

found to inhibit the synthesis of liver glycogen to the extent of −30%. A marked rise in phosphopyruvic acid was noted, which suggests another site of action for the drug. The drug was found to inhibit the anaerobic glycolysis of the rat brain homogenate to the extent of 50% inhibition with a concentration of 3.6×10^{-4} M Parahexyl.

A metabolite of chlordane in tissues of animals. BERNARD DAVIDOW, ERNEST C. HAGAN AND JACK L. RADOMSKI (introduced by ARNOLD J. LEHMAN). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

To study the tissue distribution of technical chlordane, rats were fed a diet containing 500 ppm of the insecticide. When liver, kidney, and fat were analyzed for technical chlordane, (DAVIDOW, *JAOAC* 33: 886, 1950), the fat was found to produce the most intense color. However, the absorption spectrum of this colored reaction product was different from that of the technical chlordane. Several of the individual components of technical chlordane (heptachlor, 62.5 ppm; trichloro 237, 250 ppm; and alpha and beta chlordane, 250 ppm) were then fed to rats. A study of the absorption spectra of the colored reaction product of the individual components, and of the material obtained from fat, indicates that the components of technical chlordane are stored as a metabolite. The absorption spectrum of the colored reaction product of the metabolite of each compound fed appeared identical. With trichloro 237 the original compound was stored in addition to the metabolite. In further studies heptachlor was fed daily to dogs at doses of 1, 3, and 5 mg/kg. and to rabbits at 5 and 20 mg/kg. The absorption spectra of the colored reaction product of the metabolite obtained from both dogs and rabbits were identical to that found in the rats. On the lower doses only the metabolite was found in the fat, while on the higher doses both metabolite and heptachlor were present. However, only the metabolite was found in the livers.

Serum cholinesterase changes following electro-shock. JOHN EMERSON DAVIS. *Univ. of Arkansas Med. School, Little Rock.*

Serum cholinesterase activities were determined on human subjects before and after electro-shock therapy. Activity of the enzyme was measured by an electrometric titration method which has been described previously (*Am. J. Digest. Dis.* 15: 52, 1948). It was found that no significant change was observable at 30 minutes and at 4 hours after the shock therapy. However, on the 4th–8th day following shock treatments, increases of 10–31% in cholinesterase activities were found to exist. One patient showed gradual increases following 4 successive shock treatments, involving a total increase of 46% in the period of one month. Interpretation of the data is not being attempted at this time, as the work is still in progress.

Further evidence of cholesterol-ascorbic acid antagonism in blood; role of the adrenocortical hormones. FRANCES MAE DENT,* RAYMOND L. HAYES* AND WALTER M. BOOKER. *Depts. of Pharmacology and Oral Medicine, Howard Univ. Colleges of Medicine and Dentistry, Washington, D. C.*

Acute and chronic studies have been reported recently from these laboratories describing the depression of plasma ascorbic acid by cholesterol in dogs and in rats. Urinary ascorbic acid increased concomitantly. In view of the known ability of rabbits to synthesize ascorbic acid in fairly high quantities, it was thought interesting to determine whether or not cholesterol, administered daily could consistently depress the ascorbic acid of such animals; and more important to determine whether daily administration of ascorbic acid along with cholesterol could prevent elevation of the serum cholesterol; or could the high serum cholesterol, once established, be depressed by ascorbic acid. Also of importance was to determine the extent to which adrenocortical hormones (ACE) acted in either aiding the depression of ascorbic acid by cholesterol or vice versa. Rabbits weighing 2–3 kg. were given daily 500 mg/kg. cholesterol by stomach tube. When blood levels of cholesterol had increased significantly, the animals were given 250 mg/kg. ascorbic acid daily in addition to the cholesterol. In a second group of experiments cholesterol and ascorbic acid were given initially and throughout the course of the experiment. Cholesterol-treated animals without ascorbic acid served as controls to this group. In a third group of experiments, animals which had been on cholesterol-ascorbic acid regimen, were continued, and were given 10 DU of adrenocortical hormone (extract) per kg. of body weight. Following 2 weeks on this regime, either ACE or ascorbic acid was removed. Cholesterol and cholesterol + ascorbic acid treated animals served as controls for this group.

Atypical dermatoses produced by certain agents in dermal preparations. JOHN H. DRAIZE, ELSIE ALVAREZ* AND MARIAN V. FREEMAN.* *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

An ever increasing armamentarium of chemicals is being offered in the compounding of preparations intended for topical skin and mucous membrane use. Among such products are surface active agents, antioxidants, antiseptics and vehicle bases. In the investigation of the dermal toxicity,

local and systemic effects of such products, atypical dermatoses were observed. The nature of the local skin lesions was such that the reactions could not be characterized as true primary irritation or sensitization. Albino rabbits were used for topical skin applications, and guinea pigs used for determinations of potential sensitization (DRAIZE *et al. J. Pharmacol. & Exper. Therap.* 82: 377, 1944). Atypical dermatoses resulted from dermal application of certain surface active agents producing devitalized skin with many small foci of dermal infection. Certain antiseptics (e.g. dihydroxy hexachlorodiphenyl methane (G-11)) produced sharply defined areas of intense erythema alternating with areas of severely blanched skin. Another atypical dermatitis was produced by an aluminum chloride-hydroxide complex. This latter reaction appeared first to simulate a true sensitization, unlike such allergic manifestations it disappeared with continued administration. Another type of dermatitis, obtained with hydroquinone (an antioxidant), was characterized by pin-point areas of erythema in skin otherwise exhibiting no abnormal changes.

Inhibition of cholinesterase by dimethylamino fluorophosphate. KENNETH P. DUBOIS AND KENNETH W. COCHRAN.* *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Previous studies in this laboratory demonstrated a cholinergic action by certain phosphoramides. Octamethyl pyrophosphoramide was shown to exhibit a predominant inhibitory effect on peripheral cholinesterase after conversion to an anticholinesterase agent by the liver. Phosphoramides containing ethoxy linkages exerted predominantly peripheral cholinergic effects without conversion by the liver. These findings stimulated our interest in studying fluorophosphates containing amidophosphate linkages replacing the alkoxy linkages in di-isopropyl fluorophosphate (DFP). For the present investigation bis dimethylamino fluorophosphate (supplied by Dr. Gerhardt Schrader) was employed. This compound resembled DFP in toxicity to mammals with the approximate LD_{50} for rats being 3 mg/kg. for males and 5 mg/kg. for females. It produced 50% inhibition of the cholinesterase activity of rat brain and submaxillary gland at a concentration of 4×10^{-5}M. After parenteral administration to rats maximal symptoms consisting mainly of peripheral effects appeared in about 2 hours following doses around the LD_{50}. At death (about ½ hour) after 15 mg/kg. intraperitoneally the cholinesterase activity of rat brain, submaxillary gland and serum was inhibited 34%, 82%, and 95% respectively. In 5 hours after 2.5 mg/kg. (½ LD_{50} for female rats) brain cholinesterase activity was inhibited 6% while submaxillary gland and serum were inhibited 79% and 94%, respectively. Six days later the cholinesterase activity of submaxillary gland remained 47% inhibited and serum showed 40% inhibition. These experiments indicated that dimethylamino fluorophosphate is an effective anticholinesterase agent with a long duration of action and a more prominent effect on peripheral than on brain cholinesterase *in vivo*.

Antagonism of ferricyanide to relaxation of intestinal smooth muscle caused by musculotropic drugs. GEORGE W. EDMONSTON,* PAUL F. EDGAR* AND JAMES C. RICE. *Univ. of Mississippi, University.*

Sodium ferricyanide and sodium ferrocyanide, 10^{-4}M, nullified the relaxation of upper intestinal segments of white rats caused by sodium cyanide, 10^{-4}M, and sodium azide, 10^{-4}M. The effects were studied in oxygenated Tyrode's at 37°C. A similar antagonism to the relaxation caused by CO (10^{-4}M) in the dark was observed when a larger concentration of ferricyanide, 10^{-3}M, was added to the muscle bath. Ferricyanide exerts its actions more positively than does ferrocyanide. Depression of the intestinal segments caused by sodium nitroprusside, 10^{-5}M, was antagonized effectively by even lower concentration of ferricyanide, a disparity which can be explained by the fact that the effect caused by nitro prusside is intrinsically brief. Amyl nitrite, 10^{-4}M, and nitroglycerin, 10^{-6}M, caused strong relaxations, the brevity of which required simultaneous exhibition of the relaxing drugs and ferricyanide, 10^{-6}M, to demonstrate the antagonism. Although the antagonism of ferricyanide to the relaxing actions on rat intestine caused impressively by sodium nitrite, nitrosyl chloride, hydroxylamine, and hydrogen sulfide in appropriate concentration was demonstrated at will, the observations are not competent to support the concept that ferrocyanide-ferricyanide antagonizes the effects of these intestinal relaxing drugs by reactivating, in some fashion, the cytochrome system poisoned by them. This follows because ferricyanide reacts oxidatively with sodium nitrite, hydroxylamine, and hydrogen sulfide; and because nitrosyl chloride probably distorts pH relations of the solution in muscle bath. Contrarily, the concept could satisfactorily explain the antagonism of ferricyanide against nitroglycerin, amyl nitrite, nitroprusside, and carbon monoxide in the dark.

Nicotine and the rabbit's isolated intestine. SYDNEY ELLIS AND HOLGER RASMUSSEN.* *Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Pa.*

Our experiments indicate that the isolated intestine of the rabbit (Magnus preparation) differs from the isolated intestines of other animal

species (guinea pig, cat, dog) in that its response to low concentrations (10^{-6}–10^{-5}) of nicotine is not prevented by concentrations of atropine as high as 10^{-5}. This fact makes the rabbit intestine a poor tissue for analyzing the site of action of stimulating drugs since a drug which stimulates both in the presence and absence of atropine may or may not be a 'musculotropic' agent. The action of nicotine on the rabbit's intestine appears to be an action related to nervous structure in view of the complete blockade of this action by either cocaine (affecting nerve) or *d*-tubocurarine (ganglionic blockade). Concentrations of cocaine or *d*-tubocurarine which prevent nicotine action do not interfere with the action of acetylcholine or potassium chloride. If in addition to stimulating cholinergic fibers nicotine activated adrenergic nerves which stimulate the intestine, then the rabbit intestine would be similar to the isolated muscularis mucosae of the dog (KING AND ROBINSON. *Am. J. Physiol.* 143: 325, 1945). Adrenolytic agents (dihydroergotamine, SY-28, C7337, high concentrations of ephedrine) do not inhibit the stimulating action of nicotine on the rabbit's intestine. When both an adrenolytic agent and atropine are present in the bath, the action of nicotine is antagonized. Combinations of an adrenolytic agent and atropine are not general spasmolytics as demonstrated by the persistence of the stimulating actions of potassium and of substance 'P' (V. EULER AND GADDUM. *J. Physiol.* 72: 74, 1931). The application of the above findings to explain the inability of atropine to prevent certain effects of stimulating 'parasympathetic' nerves is being investigated.

Experimental 'psychomotor' seizures and antiepileptic drugs. G. M. EVERETT AND J. E. P. TOMAN. *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

It has been shown (*EEG Clin. Neurophysiol.* 3, 1951. In press) that paroxysms of abnormal behavior resembling clinical psychomotor seizures may be induced in rats, rabbits, and mice by low frequency stimulation of the cerebral cortex. The attacks consist of approximately 20 seconds of chewing, pawing, catatonia, or other automatisms without reflex loss, and with high voltage slowly repeated sharp waves in the EEG. An assay for potential antipsychomotor drugs has been developed in mice. Stimulus parameters are set arbitrarily to give an ED_{50} for 400 mg/kg. of Phenurone. One-msec. retangular pulses at a frequency of 6 per second are delivered for 3 seconds through corneal electrodes. A current of 50 ma. (approximately 4 times threshold) is used. Over 100 drugs have now been tested orally. Phenurone is preeminent in ability to block 'psychomotor' seizures at non-depressant dosage levels, Mesantoin is less effective, Tridione acts only at depressant levels, Phenobarbital is relatively inactive, while Dilantin prolongs and modifies the attacks without abolishing them. A few new drugs were specifically effective against 'psychomotor' seizures but inactive against Metrazol and supramaximal electroshock convulsions. The data are in line with clinical findings. Psychomotor epilepsy is generally resistant to medication but is often controlled by Phenurone and to a lesser extent by Mesantoin, infrequently by other drugs. The present experiments confirm the unique nature of these seizures and suggest their usefulness in selecting drugs for clinical trial.

Change in sulfhydryl groups during urease action. H. E. EVERT (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

The variation of SH groups during the course of crystalline urease action on urea has been studied by making concurrent determinations of SH groups and urease activity. The SH groups were determined by the method of Flesch and Kun (*Proc. Soc. Exper. Biol. & Med.* 74: 249, 1950) using the red SH reagent, 1-(4 Chloromercuriphenylazo) 2-Naphthol and the urease activity was obtained by direct Nesslerization after urease inactivation with 0.5 N HCl. The experiments were carried out at 40°C. with phosphate buffer (pH 7.15, 0.2 M). The SH reagent was standardized against glutathione. In control experiments, no apparent SH change was noted with urea, phosphate buffer, sucrose or glycine. The total urea concentration was varied from 0–170 μg. in the reaction mixture. At each given urea substrate concentration, there is an initial disappearance of SH group followed by a constant value at equilibrium. The initial uptake of SH groups parallels the urease activity, and is probably involved in a urease-urea complex formation. Additional urea added to the reaction mixture after equilibrium had been reached resulted in renewed urease activity with appreciable SH change. The complex is maintained at equilibrium. In a series of experiments with increasing urea substrate concentrations, the SH level became progressively higher at equilibrium for each given initial urea concentration. However, a maximum for the SH level was reached, and further increase in initial urea concentration resulted in a decrease of SH at equilibrium.

Some effects of mercurial diuretics on renal sodium excretion in the dog. A. FARAH, T. C. COBBEY* AND W. MOOK.* *Depts. of Pharmacology and Medicine, Univ. of Washington, Seattle, and State Univ. of New York, Syracuse.*

The maximum depression of tubular sodium

reabsorption obtained with mercurial diuretics is about 2 mEq. of sodium. This confirms the findings of Duggan and Pitts (*J.C.I.* 24: 365, 1950). Sodium load is an important factor in obtaining this maximum depression. With low sodium loads the depression is less than with high sodium loads. When the sodium load is low, (infusion of hypotonic saline or glucose) or high (hypertonic saline) mercurial diuretics depress both glomerular filtration (GFR) and renal plasma flow (RPF). When isotonic saline is infused GFR and RPF are increased. When urinary sodium concentration (UNa) is low, mercurial diuretics can increase UNa above that of plasma. When UNa is high, these diuretics depress UNa but not always to plasma levels. The above data do not support the theory that mercurials act on the distal tubular mechanism. Following mercurial diuretics UNa reaches its maximum more rapidly than water excretion. BAL inhibits the increase in water excretion more rapidly than the increase in UNa. With carefully graded quantities of BAL it is possible to depress selectively the mercury-induced water excretion while UNa shows only minimal changes. Further administration of BAL will then reduce the mercury-induced increase in UNa.

Rapid determination for tocopherol in plasma. MILTON FARBER,* A. T. MILHORAT AND HARRIS ROSENKRANTZ.* *Depts. of Psychiatry and Medicine, Cornell Univ. Med. College and New York Hosp., New York City.*

The two commonly employed chemical methods for the estimation of serum or plasma tocopherol possess features which make them inconvenient for routine laboratory use. The macro method of Quaife and co-workers (*J. Biol. Chem.* 156: 499, 1944; 159: 663, 1945) utilizes hydrogenation for removal of interfering carotenoids, but hydrogenation is both time-consuming and forbidden in most hospital laboratories. A micro method (QUAIFE, SCRIMSHAW AND LOWRY. *J. Biol. Chem.* 180: 1229, 1949) which employs absorption at 460 mμ to obtain a correction for carotene interference is excellent for small quantities of blood, but is time-consuming and requires special apparatus and techniques. We have found it possible to utilize carotene absorption at 490 mμ in the ordinary photoelectric colorimeter to correct for carotene interference. The procedure involves ethanol deproteinization of a 1-to 3-ml. plasma sample, extraction and finally an Emmerie-Engel reaction reading at 515 mμ. The amount of reducing material is then calculated (as α-tocopherol) from the corrected density at 515 mμ. A series of plasma tocopherol determinations were run on 46 plasma samples by this method and by the micro method, each in duplicate, and the average values for the samples were 1.09 and 1.12 mg. %, respectively, with the mean percentage deviation between the two methods being 10.1. Tocopherol levels found in patients with muscular dystrophy and other muscular diseases show no significant abnormalities.

Sedative and toxic effects of several chloral derivatives. J. K. FINNEGAN, P. S. LARSON, H. B. HAAG AND S. G. PAGE, JR. *Dept. of Pharmacology, Med. College of Virginia, Richmond.*

The acute oral toxicity and sedative activity of the following 7 compounds have been studied in the rat: bromo-trichloroethyl-ethyl malonate (SC-1383); 2-(trichloromethyl)-4-chloromethyl-1, 3-dioxolane (SC-1400); 2-(trichloromethyl)-1,3-dioxolane-4-carbinol-acid succinate ester (SC-1433); 2-(trichloromethyl)-4-methyl-1,3-dioxolane (SC-1690); 2,7-bis (trichloromethyl)-4-methyl-1,3,6-trioxepane (SC-1781); chloral-acetamide (SC-1783) and chloral hydrate. The acute oral LD_{50}'s of aqueous solutions or suspensions of these materials were: SC-1383, 2.6 gm/kg.; SC-1400, 1.0 gm/kg.; SC-1433, 3.4 gm/kg.; SC-1690, 0.38 gm/kg.; SC-1781, > 10 gm/kg.; SC-1783, 3.1 gm/kg and chloral hydrate, 0.5 gm/kg. All compounds produce some degree of CNS depression at near-lethal levels, this being most pronounced with SC-1433 and SC-1690. Chronic feeding experiments of 8 weeks' duration were done in rats with 3 of the compounds. SC-1383 depressed growth at an intake level of 0.71 acute LD_{50}'s/day; SC-1400 at a level of 2.0 acute LD_{50}'s/day and chloral hydrate produced no effect on growth at 2.1 acute LD_{50}'s/day (maximum intake level). Histopathologic studies were negative. Sedative and toxic effects of 2 of the compounds were studied in 10 human volunteers using oral doses of 250–750 mg. SC-1781 produced a mild but variable degree of sedation in most subjects at the higher doses. Toxic effects consisted mainly of mild dizziness, headache, belching and flatulence. SC-1783 was more potent as a sedative but produced such severe headache in some of the subjects that further clinical trials were not made. (Aided by a grant from G. D. Searle & Company.)

Capacity of brain homogenates from mice infected with Columbia SK and ectromelia viruses to reduce triphenyltetrazolium chloride. JEROME T. FISHGOLD (introduced by WINDSOR C. CUTTING). *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco.*

The capacity of mouse brain homogenates to reduce triphenyltetrazolium chloride was measured by the method of Kun and Abood (*Science* 109: 144, 1949). The system was reenforced with 10^{-2}M bicarbonate and with 3×10^{-7}M methylene

blue, since these additions gave increased rates of dye reduction. Substrate concentration was 10^{-2}M Mice were injected with suspensions of the virus intraperitoneally, and were selected for study when they appeared moribund—usually within 3-6 days. Mouse brains were homogenized in isotonic sucrose, using a Potter homogenizer. Homogenates prepared from brains of mice injected with ectromelia virus showed no significant difference in their capacity to reduce triphenyltetrazolium chloride than did homogenates from control animals when the substrates were succinate, pyruvate, or glutamate. Homogenates prepared from brains of animals infected with Columbia SK virus showed capacities to reduced triphenyltetrazolium choride as follows.

SUBSTRATE	MICROGRAMS DYE REDUCED/MG. BRAIN (WET WEIGHT)/HOUR	
	Control	Infected
Pyruvate	2.6	2.0
Glutamate	2.6	1.8
Succinate	3.2	3.2

Conclusion: Brains from mice infected with Columbia SK virus have a reduced capacity to reduce triphenyltetrazolium chloride in pyruvate and glutamate substrates, but not in succinate.

Comparison of chronic effects produced in rats by several chlorinated hydrocarbon insecticides. O. G. FITZHUGH AND A. A. NELSON. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

For this presentation only the chronic feeding to rats of certain commercially important chlorinated insecticides is considered. Lifetime tests were made with DDT, TDE, toxaphene, methoxychlor, chlordane, technical benzene hexachloride (BHC) and its alpha, beta and gamma isomers. Shorter experiments were conducted with aldrin and dieldrin. The same rat diet was used in all experiments and, except for BHC, the same rat strain. Results show that beta BHC and chlordane produced the greatest damage in the long-term experiments. Liver weights were increased at 10 ppm for beta BHC and chlordane, at 25 ppm for toxaphene and at from 50–100 ppm for DDT and the alpha, gamma and technica BHC. For TDE and methoxychlor it required more than 100 ppm to affect organ weight. Varying degrees of hepatic cell enlargement, with the peripheral migration of granules occurred with all these compounds. Minimal effects occurred with 2½ ppm chlordane, 5 ppm DDT, 10 ppm beta BHC, 25 ppm toxaphene, 50 ppm alpha and gamma BHC, 100 ppm TDE and somewhat greater amount for methoxychlor. Body weight changes were not marked but occurred from 100 ppm beta BHC to greater dosage levels for the other compounds. Beta BHC showed the greatest effect on mortality. A limited amount of data indicated that aldrin and dieldrin are as toxic as chlordane. The significance of the results presented is apparent when it is realized that our human diet contains small amounts of insecticides.

Toxicity studies on amberlite XE-96, a carboxylic type cation exchange resin. THOMAS L. FLANAGAN, MARY F. SAX AND ARTHUR E. HEMING (introduced by EDWIN J. FELLOWS). *Research Div., Smith, Kline and French Labs., Philadelphia, Pa.*

Feeding experiments in which three successive generations of rats were fed the ammonium salt of Amberlite XE-96, a polycarboxylic cross-linked cation exchange resin, at a level of 10% of the diet showed no evidence of vitamin or nutritional deficiency as judged by growth curves, ability to reproduce, and histopathological examination. Rats in all three generations have shown a normal red cell count, hematocrit, hemoglobin, and white cell count indicating that there had been no interference with the hemopoietic system. Dogs receiving 30 gm. (total dose) of a mixture of ammonium and potassium salts of Amberlite XE-96 daily, mixed with their food, over a period of 6 months maintained their weight, remained in good health and showed no side effects or other signs of toxicity. A group of rats fed 10% Amberlite XE-96 in a salt-free diet for 6 months grew as well as the control rats on the salt-free diet alone. Histopathological examination of tissues from all of the above animals was completely negative.

Inhibition of the hydrolysis of procaine and 2-chloroprocaine in plasma by neostigmine and dimethylcarbamate of (2-hydroxy-5-phenylbenzyl) trimethyl-ammonium bromide (NU-683). FRANCIS F. FOLDES AND MANUEL H. AVEN (introduced by L. O. RANDALL). *Dept. of Anesthesia, Mercy Hosp., and Dept. of Anesthesia, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

It has been well known that both neostigmine and NU-683 possess marked anticholinesterase activity. It was found that in this respect NU-683 was one-tenth as potent as neostigmine (Jubilee Volume *Emil Barell.* 1946, p. 315). Kisch has demonstrated (*Exper. Med. & Surg.* 1: 84, 1943) that neostigmine in a 1:100,000 concentration markedly decreased the procaine esterase activity of human serum. Incubating at 37°C. human plasma samples containing 0.1 mg/cc. of procaine for 15 minutes, or 0.1 mg/cc. of 2-chloroprocaine for 4 minutes with or without the addition of various concentrations of neostigmine or NU-683, we found that the addition of 1:100,000 of neostigmine decreased the hydrolysis of procaine from an average of 90% to an average of 15%, and the hydrolysis of 2-chlorprocaine from an average of 100% to an average of 15%. In a 1:1,000,000 con-

centration the inhibitory effect of neostigmine was reduced by 50%. NU-683 in dilutions up to 1:1,000,000 decreased the hydrolysis of procaine from an average of 90% to an average of 7%, and the hydrolysis of 2-chloroprocaine from an average of 100% to an average of 10%. NU-683 was found to markedly inhibit the hydrolysis of procaine and 2-chloroprocaine even in concentrations of 1:10,000,000. Our experiments indicate that the inhibitory effect of NU-683 on procaine esterase activity is at least ten times greater than that of neostigmine. The marked difference between the anti-chlolinesterase and the anti-procaine esterase activity of NU-683 is an added proof against the identity of the two enzymes.

Circulatory and cardiac metabolic changes following intravenous injections of protoveratrine. E. L. FOLTZ, R. G. PAGE, C. W. CRUMPTON AND J. ALANIS. (introduced by PAUL R. DUMKE). *Robinette Fndn., Hosp. of Univ. of Pennsylvania, and Lab. of Pharmacology, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

In normal dogs under our standard anesthesia (morphinedial-urethane-pentobarbital) intravenous injection of 2.5–3.0 μg. protoveratrine per kg. produced a prolonged fall in mean blood pressure and bradycardia, followed occasionally by tachycardia, and moderate slowing of breathing. Coronary blood flow (nitrous oxide-coronary sinus catheterization method) was decreased, as were cardiac oxygen consumption, coronary venous oxygen saturation and cardiac work. Coronary vascular tonus and cardiac efficiency were not significantly altered. These findings do not indicate a therapeutically useful effect of protoveratrine on the coronary circulation and cardiac oxygen metabolism under these experimental conditions.

Effect of procaine, epinephrine, cardiac massage and electrical countershock on ventricular fibrillation. CHARLES FRANK,* GEORGE MISRAHY,* HSUEH H. WANG,* ROBERT MILLER* AND RENÉ WÉGRIA. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Presbyterian Hosp., New York City.*

In 65 dogs anesthetized with sodium pentobarbital, the chest was opened and, under artificial respiration, the heart was exposed and suspended in a pericardial cradle. Arterial blood pressure and electrocardiogram were recorded. Ventricular fibrillation was induced electrically. Neither cardiac massage alone nor intracardiac epinephrine alone stopped ventricular fibrillation. Countershock applied after 30 seconds of fibrillation restored coordinated ventricular contractions and the blood pressure returned to or toward control. When applied after 5 minutes of fibrillation, countershock restored coordinated ventricular contractions but these were too weak to raise the blood pressure. Countershock remained effective in restoring coordinated competent ventricular contractions when applied after 10–15 minutes of fibrillation, provided cardiac massage was applied during this interval. The intraventricular injection of procaine after 30 seconds of fibrillation always slowed the fibrillation rate with all the doses tried. Doses of 200 mg/kg. of body weight produced ventricular standstill. With doses of from 20–150 mg., standstill occurred in half of the experiments. Weak coordinated ventricular contractions followed standstill only with doses of 50 mg. or less. Cardiac massage applied to such weakly beating hearts resulted in ventricular fibrillation except in one dog in which vigorous contractions and adequate blood pressure were restored. Apparently the dose of procaine required to stop fibrillation was usually sufficient to prevent the development of competent ventricular contractions.

Cortisone therapy in barbiturate abstinence syndrome. H. F. FRASER,* H. ISBELL, A. WIKLER, A. J. EISENMAN AND C. H. KORNETSKY.* *Research Branch, U. S. Public Health Service Hosp., Lexington, Ky.*

Patient A was actively addicted to barbiturates and opiates. During his current hospital admission he was given only minimal amounts of pentobarbital during morphine withdrawal. A withdrawal-type grand mal convulsion appeared on the 12th day. Secobarbital was then administered in increasing doses to a level of about 2.5 gm. daily, and maintained from the 21st through the 45th day. No significant symptoms of intoxication developed. A withdrawal psychosis consisting of confusion, visual and auditory hallucinations occurred on the 15th and 16th days, with a mild recurrence on the 25th day. On the 46th day, secobarbital was discontinued abruptly. Cortisone, 100 mg., was given intramuscularly 24 and again 5 hours prior to withdrawal. After 18 hours abstinence, anxiety, pass-pointing and tremors developed. Cortisone dosage was increased to 300 mg. daily and symptoms were relieved. Cortisone was continued for 2 weeks in a total dose of 2325 mg. He improved progressively and showed no signs of convulsions or psychosis and was discharged on the 66th day. *Patient B* was actively addicted to alcohol, opiates and barbiturates. His hospital course was analagous to *A* except that secobarbital was discontinued abruptly and no cortisone was administered. After 46 hours of abstinence, a grand mal convulsion appeared. Cortisone therapy was then instituted and a total of 1400 mg. was given in 5 days. No further seizures appeared but psychosis developed on the 3d day of treatment and

persisted for 3 days, following which progressive recovery occurred. Further clinical electroencephalographic, psychological and biochemical studies are in progress.

Rate of recovery of plasma, red cell and brain tissue of rats following cholinesterase inhibition by organic phosphates. JOHN P. FRAWLEY AND ERNEST C. HAGAN (introduced by O. G. FITZHUGH). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

The effect of DFP (diisopropyl fluorophosphate), parathion (0,0-diethyl, 0-p-nitrophenyl thiophosphate), EPN (ethyl p-nitrophenyl thionobenzene phosphonate), OMPA (Octamethyl pyrophosphoramide), TEPP (tetraethyl pyrophosphate) and E-838 (diethoxy thiophosphoric acid ester of 7-hydroxy-4-methyl coumarin) on the cholinesterase activity of rat blood, red cells and plasma, and of brain tissue has been studied. Cholinesterase determinations were made by a modification of the electrometric procedure of Michel (*J. Lab. & Clin. Med.* 34: 1564, 1949), for human blood. Following a single dose of 1 mg/kg. of TEPP, 5 mg/kg. of parathion, DFP, or OMPA, 15 mg/kg. of E-838 or 25 mg/kg. of EPN, the plasma activity was depressed below 50%, but returned to normal within 3 days. Cell activity was also depressed below 50% of normal with each compound but required more than a month for complete recovery. OMPA had no effect on brain cholinesterase. All other compounds except DFP caused brain cholinesterase depression which was completely reversible within one week. DFP caused a depression of brain activity which did not recover for more than one month. Data showing the cumulative effect on cell cholinesterase of very small doses of these compounds will also be presented.

Chronic tolerance studies on a new hydrocholeretic, β-(2,4-dimethoxy-5-cyclohexylbenzoyl) propionic acid (SC-2644). HOMER B. FREESE,* W. E. HAMBOURGER AND D. M. GREEN. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

Tolerance studies on β-(2,4-dimethoxy-5-cyclohexylbenzoyl) propionic acid (SC-2644), a new hydrocholeretic (COOK *et al. J. Pharmacol. & Exper. Therap.* 100: 421, 1950) were conducted in rats and dogs to determine whether repeated daily administration would produce evidence of toxicity. Doses for these experiments were based on results of preliminary studies over wider dosage ranges. Three groups of 12 male and 12 female rats each received SC-2644 dissolved in drinking water in such concentration as to provide daily amounts of approximately $\frac{1}{4}$, $\frac{1}{8}$ or $\frac{1}{16}$ the acute mouse intragastric LD_{50} (1020 mg/kg.). A control group received no drug. The study has been continued into the second and third generation. There have been no spontaneous deaths. At the highest doage level growth was retarded; the offspring were smaller in size. First generation rats sacrificed at 31 and 114 days had no lesions due to SC-2644. Two groups of 8 dogs each were given capsules containing 64 or 32 mg/kg., administered 5 days each week. Hepatic, hematologic and urinary studies have remained essentially normal. Occasional instances of emesis and diarrhea occurred at both doses. Four dogs sacrificed at 31 and 4 at 107 days had no lesions attributable to SC-2644. No spontaneous deaths occurred in the remaining 8 which have now been under treatment more than 28 weeks.

Action of desoxyephedrine on the vascular bed of the limb of the dog. M. JACK FRUMIN,* SHIH-HSUN NGAI* AND E. M. PAPPER. *Dept. of Anesthesiology (Surgery), Columbia Univ. College of Physicians and Surgeons, New York City.*

The action of intra-arterial epinephrine, nor-epinephrine and desoxyephedrine on the vascular bed of the hind limb of the dog was investigated by the direct measurement of the femoral arterial blood flow with a recording rotameter. The maintenance of a stable arterial pressure permitted the interpretation of flow rate variations as either vasoconstriction or vasodilatation. Changes in the degree of vasomotor tone were inferred from the quantitative changes in flow rate. The effects of these amines were noted in four types of preparations: *1*) the resting anesthetized state, *2*) bilateral common carotid artery occlusion, *3*) segmental peridural block, *4*) adrenergic blockade with DHE-45 or Dibenamine. Initial doses of desoxyephedrine in the first three preparations caused a temporary dilatation which paralleled the existing vascular tone and was followed by prolonged constriction. Subsequent doses of desoxyephedrine caused dilatation, either completely or predominantly. Nor-epinephrine always produced constriction, while epinephrine caused constriction, either entirely or predominantly. After adrenergic blockade, however, desoxyephedrine and nor-epinephrine produced no effect, and epinephrine caused only dilatation. It was concluded that the action of desoxyephedrine was the competition with the sympathetic mediator for receptors on the effector cells and the interference with the destruction or elimination of the mediator.

Metabolism of 8-azaguanine in rabbits. ALFRED GELLHORN, JACOB KREAM* AND ERICH HIRSCHBERG.* *Cancer Research Inst., Columbia Univ.*

College of Physicians and Surgeons, New York City.

Studies in this laboratory and elsewhere have demonstrated that the guanine analog, 8-azaguanine, significantly inhibits the growth of certain tumors in experimental animals whereas others are unaffected. In addition, evidence has been presented which shows that cell division in susceptible tumors is depressed while in testicular epithelium and intestinal crypts of the same animals no detectable alteration of mitotic rate occurs. These observations indicate that 8-azaguanine may be a useful tool for studying biochemical differences between morphologically separable tumors and between neoplastic and normal cells. *In vitro* experiments here have demonstrated the presence in various tissues of a deaminating enzyme which converts 8-azaguanine to the non-carcinostatic 8-azaxanthine. The cellular concentration of this deaminase is low in an 8-azaguanine susceptible tumor as compared with a non-susceptible tumor and several normal tissues. Evidence will be submitted which indicates that these *in vitro* observations are relevant to the *in vivo* metabolism of 8-azaguanine. The urinary excretion of 8-azaguanine and 8-azaxanthine has been determined in rabbits following intraperitoneal administration of 8-azaguanine. The separation as well as the qualitative and quantitative determination of these two substances has been accomplished by paper partition chromatography of urine aliquots, using an *n*-butanol-diethylene glycol-0.1 N HCl mixture as the solvent, and elution of the spots with appropriate solvents. The spectral characteristics of the eluates compare well with the spectra of authentic samples of 8-azaguanine and 8-azaxanthine. The results thus far indicate that the major metabolic product of parenterally administered 8-azaguanine is 8-azaxanthine.

Quinidine, auricular excitability and fibrillation. J. L. GILBERT,* A. A. SIEBENS,* B. F. HOFFMAN,* J. BELFORD,* H. M. WOSKE* AND C. McC. BROOKS. *State Univ. Med. Center at New York City.*

Excitability changes and fibrillation thresholds of the dog's auricle were determined (*Am. J. Physiol.* 162: 219, 1950). Quinidine (7.5–15 mg/kg.) was administered intravenously. Two- or three-msec. duration test shocks were used to ascertain changes in susceptibility to fibrillation, coincident modifications in refractoriness, diastolic excitability, the 'dip,' conductivity, latency, and the QT interval. In 14 representative experiments total refractoriness was prolonged by approximately 50 msec., the absolute refractory period by 20 and the relative by 30 msec. Increases in diastolic threshold were definitely greater (up to 40%) than spontaneous variations. As the relative refractory period lengthened to occupy a greater proportion of the cycle, thresholds within this period were frequently elevated, thus changing the shape of the recovery curve. The 'dip' was not eradicated but maintained its relative position. Inter-electrode conduction times were increased by about 10%, QT interval and latency by approximately 20 msec. The minimum blood quinidine level producing these changes and abolishing fibrillation was 3 mg./liter. The prolongation of the total refractory period was the most marked change coincidental with fibrillation eradication. However, there were enough instances in which the boundaries of the total refractory period were extended without any change in fibrillation susceptibility, and instances in which fibrillation was abolished without much prolongation of the refractory period to cast doubt upon the concept that an increase in absolute or total refractory period is the sole cause of the antifibrillatory action of quinidine.

A comparison of effect of various barbiturates on contraction of skeletal muscle. MELVYN I. GLUCKMAN* AND CHARLES P. KRAATZ. *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

Maximal twitches were recorded isotonically from the isolated frog sartorius muscle and isometrically from the dog anterior tibial muscle *in situ*. The sartorius muscle, stimulated directly, was irrigated with Ringer's solution containing barbiturates. In the dog, injections were made into the femoral artery while the anterior tibial muscle was stimulated through the sciatic nerve at a rate of 30/minute. Equimolar comparisons were made of diallyl barbituric acid and the sodium salts of thiopental, pentobarbital, amobarbital, thioethamyl, vinbarbital, butethal, secobarbital, barbital, hexethal, butabarbital, hexobarbital, phenobarbital, and aprobarbital. The height of the maximal twitch of the sartorius muscle was increased by concentrations of thiopental, thioethamyl and pentobarbital from M/6000 to M/2000. Thiopental and thioethamyl in a M/6000 solution augmented the twitch approximately 45% and pentobarbital in the same concentration about 25%. Amobarbital was less active, increasing the contraction approximately 10% in a M/2000 concentration. The other barbiturates studied showed no activity in this type of experiment. In the dog thiopental showed the greatest potentiation of the twitch followed closely by thioethamyl. Pentobarbital, amobarbital, secobarbital and hexethal were intermediate while butabarbital, aprobarbital and butethal

had only slight potentiating activity. Barbital, vinbarbital, hexobarbital, phenobarbital and diallyl barbituric acid were essentially negative.

An improved ultraviolet spectrophotometric procedure for the determination and differentiation of barbiturates. LEO R. GOLDBAUM (introduced by PAUL K. SMITH). *Army Med. Service Graduate School, Army Med. Center, Washington, D. C.*

Barbiturates have ultraviolet absorption spectra with maxima at 250 mμ and minima at 235 mμ in strong alkaline solution. In solution at pH 10 the maxima are at 240 mμ. If the optical densities of pH 10 solutions are subtracted from those obtained in strongly alkaline solution, the differences in these spectra are maximal at 260 mμ, exhibit a negative maximal difference at 240 mμ or 235 mμ becoming positive again at 225 mμ. These differences in spectra are specific for barbiturates. When comparison is made of the differences at other wave lengths with that at 260 mμ, five groups of barbiturates can be differentiated depending upon the substitutions on the *5* position of the malonylurea ring. Using the extraction procedure already described (*J. Pharmacol. & Exper. Therap.* 94: 68, 1948), the ultraviolet spectrum of the 0.5 N NaOH extract is determined in a Beckman DU-spectrophotometer. This is diluted with borate buffer (pH 7.3) to yield a solution of pH 10±0.2. The ultraviolet absorption spectrum is determined again using as a reference solution 0.5 N NaOH diluted with borate buffer. After correcting for the dilution by the buffer, the optical densities of the buffered solution are subtracted from those of the 0.5 N NaOH. Determination of the difference spectra furnishes a simple means for the estimation and identification of barbiturates in biological materials since the normal extracts have similar optical densities in alkali and in pH 10 buffer.

Cardiovascular effects of erythrophleum alkaloids. L. I. GOLDBERG* AND R. P. WALTON. *Dept. of Pharmacology, Med. College of South Carolina, Charleston.*

Erythrophleine sulfate and an extract containing the total alkaloids of Erythrophleum guineense were both found to give responses closely similar to those of the cardiac glycosides. The period for onset of effects corresponded most nearly to that of the prompter acting glycosides such as thevetin. Heart contractile force, arterial pressure, and ECG changes were determined according to procedures previously described (*J.P.E.T.* 98: 346, 1950; *Am. J. Physiol.* 161: 489, 1950). 'Cat unit' potencies of these preparations were determined directly, using the procedure of Chen *et al.* (*J. A. Pharm. A.* 25: 579, 1936). In terms of CU doses, the effects of these preparations were quantitatively similar to those obtained with the cardiac glycosides. ECG tracings showed typical S-T depression in most cases, varying degrees of A-V block, coupling, ventricular tachycardia and terminal ventricular fibrillation. As with the glycosides, contractile force increments commonly preceded significant ECG changes. Coumingine HCl produced distinct increments in contractile force but did not fully exhibit the typical action of cardiac glycosides. Although comparably severe grades of arrhythmia developed, the relative contractile force and arterial pressure increments did not appear to be as great; this is in agreement with related observations of Maling and Krayer (*J.P. E.T.* 86: 66, 1946). When the erythrophleum extract was administered by direct i.v. injection in unanesthetised dogs, the minimum dose producing emesis in 100% of trials corresponded to 0.5 CU. Concomitant experiments with ouabain gave a corresponding figure of about 0.5 CU in agreement with observations of Chen *et al.* in cats.

Physiological performance following a hypnotic dose of a barbiturate. ROBERT E. GOODNOW,* HENRY K. BEECHER, MARY A. B. BRAZIER,* FREDERICK MOSTELLER* AND RENATO TAGIURI.* *Anesthesia Lab. of Harvard Med. School at Massachusetts General Hosp., Boston.*

The after-effects of 0.1 gm. pentobarbital sodium administered by mouth were investigated by means of psycho-physiological performance tests. Four tests, representing a range of functions, *Tapping Speed, Auditory Reaction Time, Naming of Opposites, and Memory for Digits*, showed a significant deterioration in performance at 6:00 A.M., four hours after the barbiturate had been taken. The effect observed at 6:00 A.M. diminished after breakfast but was found to continue in a highly suggestive (qualitative trend) but not statistically significant degree until after 4:00 P.M., 14 hours after medication. Although previous studies had been unable to demonstrate the effects of small doses upon test performance, the present findings were made possible by controlling many of the sources of variability, by means of the experimental design and analytic procedures. This demonstration of persistent impairment by the usual dose of a barbiturate supports the view that legislation concerning the use of these agents needs tightening.

Further studies on in vitro metabolism of thiopental. THELMA C. GOULD* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

A previous report from this laboratory (GOULD,

T. C. AND F. E. SHIDEMAN. *Am. Soc. Exper. Pharm. & Therap.*, Fall Meeting, 1950, Boston) described a cell-free preparation of rat liver which, when properly supplemented, would metabolize thiopental. It was also demonstrated that this metabolic process is aerobic. In an attempt to obtain more information regarding the metabolic composition of this system the effects of various inhibitors have been tested. Sodium cyanide (2×10^{-2}M) completely inhibits the disappearance of thiopental. Dependence of the system on energy-rich phosphate bond compounds is indicated by the fact that omission of ATP from the system diminishes activity approximately 25%. Addition of 2,4-dinitrophenol at a concentration (10^{-4}M) which completely suppresses phosphorylation of creatine by a properly supplemented liver homogenate inhibits the metabolism approximately 30%. Preliminary experiments had shown that the amount of thiopental metabolized was approximately doubled by fortifying the system with certain intermediates of the Kreb's cycle—citrate, α-ketoglutarate, succinate, fumarate, or malate. Using succinate as a substrate the metabolism of thiopental was not inhibited by malonate in concentrations (5×10^{-2} M) which depressed the oxygen uptake of the system by 70%. Other inhibitors have been employed and their effects will be discussed. Attempts have been made to concentrate the activity of the liver homogenate by fractional centrifugation. By centrifuging in the cold at speeds as low as 500 rpm heavy particulate matter is thrown down which alone has as activity (based on dry weight of tissue) three to four times greater than that of the original whole homogenate. The activity of the supernatant is approximately equal to that of the whole homogenate.

Phosphate turnover in the human erythrocyte. D. R. H. GOURLEY (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

Previous reports (*J. Cell. & Comp. Physiol.* 35: 341, 1950; *Am. J. Physiol.* 163: 716, 1950) have indicated that the uptake of P^{32}-labeled phosphate ions by human erythrocytes is an active chemical process, possibly enzymatic in nature. An attempt has been made to further characterize this process by studying the time-course of the P^{32} concentrations in several acid-soluble phosphorus compounds of human blood *in vitro*. The fractionation procedure devised by Sacks for compounds of this type in liver (*J. Biol. Chem.* 181: 655, 1949) was adapted for use with blood. The rate of P^{32} turnover in the following fractions has been observed: inorganic phosphate, the labile and the stable P of ATP-ADP, adenylic acid, diphosphoglyceric acid, glycerophosphoric acid, glucose-1-phosphate, glucose-6-phosphate, coenzymes, and propanediol phosphate. It was found that the specific activity of the P in the organic acid-soluble phosphorus compounds of the cells increases at different rates. The highest specific activity is attained by the labile P of ATP-ADP although the P of diphosphoglyceric acid approaches the same value after three hours at 37° C. A measurable amount of P^{32} is also found in glucose-1-phosphate, in the stable P of ATP-ADP and in adenylic acid. Very little P^{32} enters the other compounds investigated and none enters propanediol phosphate during the three-hour period of observation. These results offer further evidence that the uptake of phosphate by human erythrocytes is intimately connected with the metabolic activity of the cells.

Effects of Veratrum alkaloids on isolated rabbit intestine. JAMES T. GOURZIS AND ROBERT O. BAUER (introduced by GEORGE MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

In a previous communication (*Proc. J. Pharmacol. & Exper. Therap.* In press) the spasmogenic and tachyphylactic effects of Veriloid (Riker Laboratories) a mixture of alkaloids of Veratrum viride on isolated rabbit intestine were reported. These observations have been extended to individual Veratrum alkaloids—Protoveratrine, Germerine, Veratridine and Cevadine. Rabbit intestine was prepared according to the method of Magnus utilizing a constant temperature bath (37.5°C.) and Tyrode's solution. Strips from at least 10 animals were exposed to the desired concentration of each alkaloid. Qualitatively the effects of all these alkaloids mimicked those of Veriloid. The predominant response to minimal effective doses was an increase in the height of segmented activity. Increase in tone was occasionally seen at such doses. Response to high doses was invariably an increase in tone. The segmental rate tended to remain constant. The ED_{50} spasmogenic dose (μg/liter bath) and 95% confidence limits were—Germerine 0.94 (0.65–1.4), Protoveratrine 2.7 (2.2–3.3), Veratridine 4.7 (4.1–5.4) and Cevadine 7.8 (6.7–9.0).

Fate of injected coenzyme A. WM. M. GOVIER AND ANNA J. GIBBONS.* *Research Div., Upjohn Company, Kalamazoo, Mich.*

The parenteral administration of coenzyme A to dogs by the intravenous route resulted in the accumulation in plasma and renal excretion of the 'potato split product' of Novelli *et al.* (*Federation Proc.* 9: 209, 1950). The coenzyme was not absorbed from the gastrointestinal tract as such or as 'potato split product.' Intravenous administration of coenzyme A to dogs resulted in accumulation of coenzyme A in adrenal, liver, and muscle tissues.

It would thus appear that parenterally injected coenzyme A is broken down to the 'potato split product' which is probably in turn resynthesized in the tissues to coenzyme A. Similar data dealing with results following injection by other routes in dogs and guinea pigs are presented.

Difference in response of normal and chronically denervated submaxillary salivary gland of cat to acetylcholine. A. R. GRAHAM* AND G. W. STAVRAKY. *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.*

It was previously shown in the acutely denervated submaxillary salivary gland of the cat that acetylcholine in low concentrations produces vasodilatation and secretion, while in high concentrations it produces vasoconstriction which is often accompanied by an inhibition of secretion. STAVRAKY AND GRAHAM. *Federation Proc.* 9: 317, 1950). In the present investigation carried out on 21 cats, chronic denervation of one gland was accomplished by aseptic excision of either the chorda tympani or of the superior cervical sympathetic ganglion. Sufficient time (10–423 days) for sensitisation by denervation was allowed after which the remaining nervous connection of the gland was severed, the control gland on the opposite side was acutely denervated, and experiments performed in which blood flow and salivary secretion were measured simultaneously from both glands by means of electronic drop-recorders calibrated to deliver drops of equal volume. The chronically denervated gland was found to be uniformly more sensitive than the control one to all concentrations of acetylcholine employed (10^{-6}–1000 μg. per 1/10 cc.). The gland had a lower vasomotor and secretory threshold and it responded by a greater vasodilatation and secretion to low concentrations of acetylcholine. High concentrations of acetylcholine produced, in the previously denervated gland, a greater vasoconstriction and a more marked inhibition of secretion than in the control gland. An analysis of the relation of the rate of secretion to the rate of blood flow in the control and denervated gland is being carried out at the present time.

Blood pH determinations: a new and accurate clinical method. FRANK GRAIG, KURT LANGE AND STEVE CARSON (introduced by M. G. MULINOS). *Dept. of Medicine, New York Med. College, New York City.*

Arterial or venous blood is collected in a heparinized syringe with a 3-way stopcock interposed between syringe and needle. The blood is then transferred into a special calibrated pipette by turning of the stopcock. This pipette was previously prepared with equal mixtures of heparin and NaFl to prevent clotting and glycolysis, respectively, but which do not influence the pH at the concentrations used. A stopcock on top of the pipette prevents aeration of the sample, and a glass bead in the bulb facilitates mixing. The samples may be kept in the refrigerator or at room temperature for as long as 24 hours. The blood is transferred anaerobically from the pipette into the glass electrode of a Cambridge Research pH meter; 3 minutes are allowed for equilibration and the result can be read at room temperature directly in pH units. The value must be corrected to 37° C. by a factor differing from that described by Rosenthal. This method has the following advantages. *1)* Ease and speed permit numerous and frequent determinations in all types of cases. *2)* The readings are accurate and reproducible within 24 hours with an error of not more than ± 0.01 pH unit. *3)* The collection of samples is simple and readings may be postponed until convenient. *4)* Use of the pH meter requires no special skill, only average laboratory training. Our studies have shown that routine pH determinations are preferable to the CO_2 combining power in evaluating acid-base disturbances.

Synergistic effect of Thenfadil [N,N-Dimethyl-N′-(3-thenyl)-N′-(α pyridyl)-ethylenediamine (sic)] and WIN 3046 [1-(3,4-Dihydroxyphenyl-2-isopropylamino-1-butanol] in guinea pigs exposed to histamine aerosols. JESSE I. GRANT (introducted by A. M. LANDS). *Biology Div., Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

The antihistaminic activities of Thenfadil and WIN 3046, singly and in combination were determined by testing their protective action in histamine-induced broncho-constriction in the guinea pig. The 'histamine asthma' technic described by Siegmund *et al.* (*J.P.E.T.* 90: 254, 1947), in somewhat modified form, was used. Guinea pigs were exposed to aerosols of varying concentration of each compound dissolved in 0.2% histamine diphosphate solution. Dose-response curves were obtained for both drugs. The results obtained with the combination has revealed a synergism. A concentration of 25 μg/ml. of Thenfadil protected <10% and 25 μg/ml. of WIN 3046 protected 25% of the guinea pigs whereas in combination 48% protection was obtained. Similarly 100 μg/ml. Thenfadil and 25 μg/ml. WIN 3046 protected 10% and 25%, respectively, whereas with drugs in combination, 66% of the animals were protected. These data indicate that combined therapy in this dosage range produced more than an additive effect.

Tachyphylaxis to inhibitory action of atropine on intestinal motility. GRACE W. GRAY* AND M. H. SEEVERS. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

During studies designed to determine the thresh-

old inhibitory dose of atropine on intestinal motility, acute tolerance (tachyphylaxis) was observed. (This phenomenon has also been reported by Quigley.) It has been demonstrated in this laboratory at all intestinal levels from jejunum through colon, using unanesthetized dogs with various types of intestinal fistulae. A completely refractory state of several hours duration can be produced by a single or several repeated injections of .2 mg. of atropine. Once the refractory state is established, motility is unaffected by doses of atropine as large as 1 mg/kg., and crossed tolerance to chemically related drugs exists. Products of mild alkaline hydrolysis of atropine, administered in quantities equivalent to 400 times the test dose, were ineffective in depressing intestinal motility or inducing the refractory state. Tolerance to atropine could not be produced by previous injection of drugs such as TEA which possess a different site of action. Thus, the phenomenon appears not to depend upon a previous state of depressed motility per se. It has also been shown to be independent of the extrinsic nerve supply of the bowel, being easily demonstrated in various chronically denervated preparations. During the refractory state, intravenous methacholine (30 μg) was completely ineffective in producing hypermotility. Resensitization to methacholine and atropine were found to occur concomitantly several hours later. The significance of these findings will be discussed.

Influence of potassium and calcium on mammalian myocardial activity within the physiological range. JACK PETER GREEN AND NICHOLAS J. GIARMAN (introduced by WILLIAM T. SALTER). *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*

Unlike the frog heart at 26° C., the contractility of isolated cat papillary muscle at 38° C. in Krebs-Henseleit solution remains essentially unaffected by changes in free potassium concentration from 3–10 mM. In order to demonstrate potassium effects on this preparation it is necessary to use non-physiological concentrations. Moreover, the response observed under such conditions represents a marked distortion of normal excitability and contractility. Like the frog heart, the cat papillary muscle shows a marked sensitivity to variations in ionic calcium. This graded response, however, is unaffected by potassium; and the traditional concept of the K:Ca ratio needs re-valuation in the non-toxic range. Unlike contractility the mammalian myocardium does exhibit alterations in excitability with changes in physiological concentrations of ionic potassium as well as calcium. An increase in ionic calcium *increases* contractility but *decreases* excitability within non-toxic limits. For potassium no relationship was demonstrated between excitability and contractility.

In vitro metabolism of pamaquine in chicken droppings. JOSEPH GREENBERG, EDWARD S. JOSEPHSON, H. L. BAMI AND D. JANE TAYLOR (introduced by N. B. EDDY). *Natl. Insts. of Health, Natl. Microbiological Inst., Bethesda, Md., and Malaria Inst. of India, Delhi.*

Pamaquine was incubated at 37° C. for 24 hours at pH 7 with chicken droppings which were then extracted with 30:70 benzene-butanol. Separation of metabolites was accomplished by chromatography on a cellulose column saturated with 1 N HCl. Five distinct products were isolated, which, in order of their rate of flow, were labelled Red I, Red II, Yellow I, Yellow II, and Yellow III. The ultraviolet absorption spectra, antimalarial activity *in vitro* and methemoglobin producing properties of these metabolites have been obtained. None of these substances coupled with diazotized sulfanilic acid and only Yellow I was reversibly reduced by ascorbic acid. Yellow I had an ultraviolet absorption spectrum of a 5,6-quinoline-quinone, and formed a quinoxaline derivative, when reacted with *o*-phenylenediamine, which was crystallized as the dipicrate, melting at 202–204° C. This compares with the dipicrate of a similar quinoxaline derivative prepared from synthetic 8-8-(diethylamino-1-methylbutylamino)-5,6-quinoline-quinone, by Drs. Eleanor Werble and R. E. Elderfield of Columbia University, with a m.p. of 202°C. None of the other products formed similar quinoxalines.

Replacement of potassium in the human erythrocyte during cholinesterase activity. MARGARET E. GREIG, THOMAS C. MAYBERRY* AND C. ELIZABETH DUNN.* *Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

We have previously shown that so long as acetylcholine was being hydrolyzed optimally by cholinesterase the internal ionic composition of the erythrocyte tended to remain constant. If cholinesterase was inactive either due to lack of substrate or because of inhibition there was a shift of sodium and potassium ions in the direction towards establishment of thermodynamic equilibrium. It has now been found that if erthrocytes are incubated in isotonic sodium bicarbonate containing about 35 mEq/l. potassium chloride until they have lost a measurable portion of their potassium, the potassium can be replaced in the cell by addition to the medium of acetylcholine or of triacetin both of which are hydrolyzed by cholinesterase. When benzoyl choline, which is not appreciably hydrolyzed by erythrocyte cholinesterase, is added, no replacement of potassium occurs, but instead the cell continues to lose potassium as is the case in the suspension without added substrate. The sodium which enters cells suspended in the above medium without substrate does not seem to be removed after adding acetylcholine. It would

thus appear that the transfer of potassium is an active process which may depend on cholinesterase activity while the transfer of sodium is passive.

Further observations on site of the emetic action of digitalis glycosides in man. THEODORE GREINER,* HARRY GOLD, HAROLD OTTO,* JOSEPH GLUCK,* SYDNEY MATHES,* RAYMOND MARSH,* DEAN HUDSON,* DONALD ROBERTSON,* WALTER MODELL, LEON WARSHAW* AND MILTON KRAMER.* *Dept. of Pharmacology, Cornell Univ. Med. College, Cardiovascular Research Unit, Beth Israel Hosp., and Cardiology Service Hosp. for Joint Diseases, New York City.*

Experiments reported at the meeting of this society last year indicated that the conventional view that vomiting from overdigitalization by the oral route in man is a systemic action may be erroneous, and that the action is more likely a local one in the gastrointestinal tract. The study has been extended to include digitoxin, digoxin, lanatoside C, and Digifoline. Patients received oral doses over a period of 24–48 hours until vomiting occurred, and after recovery, intravenous doses repeated at varying intervals. As judged by the change in the T-wave, impairment of A-V conduction, or the slowing of the ventricle in auricular fibrillation, greater cardiac effects without gastrointestinal toxicity were possible by the intravenous than by the oral route. This is taken as evidence that vomiting commonly ascribed to a systemic action is a local action, since it can be avoided by the parenteral route of administration. These results have far-reaching implications, since they point to the possibility of obtaining greater therapeutic action on the heart by parenteral than by oral administration, and to an important factor in the choice of digitalis glycosides, for in a previous publication we presented evidence showing wide differences in the local emetic activity of different members of the digitalis group.

Toxicology and anticoagulant activity of Tromexan. CHARLES M. GRUBER, JR., CHARLES M. GRUBER, KWANG SOO LEE* AND ZINAIDA T. LASZICZENKO.* *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

Lethal doses of Tromexan given orally or intraperitoneally produce death in mice, rats and some rabbits within twelve hours probably by cardiac arrest. In other rabbits delayed death, which results from hemorrhage into the caecum, was also a common sequel to these doses. The oral and intraperitoneal LD_{50} doses for mice, rats and rabbits are 0.88, 0.26; 0.84, 0.32; 1.1 and 0.17 gm/kg., respectively. During a 3-month period, no signs of chronic toxicity to orally administered Tromexan developed in rats with an average intake of 25.8 mg/kg. daily or in dogs receiving an average dose of 1.0 mg/kg. daily. In dogs, Tromexan produces hypoprothrombinemia more rapidly than does bishydroxycoumarin. The return to normal prothrombin blood levels is also more rapid. No absolute evidence of an antagonism to this hypoprothrombinemic effect of Tromexan was obtained with daily doses of 0.1 gm. of vitamin K_1 or of 2.0 mg. of menadione sodium bisulfite subcutaneously.

Effect of procaine, 'Pronestyl' and β-diethylaminoethanol on epinephrine-cyclopropane induced ventricular arrhythmias. CARL C. GRUHZIT (introduced by MAYNARD B. CHENOWETH). *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

The effect of an intravenous infusion of 1–5 mg/kg/min. procaine hydrochloride, 1–5 mg/kg/min. procaine amide hydrochloride ('Pronestyl'), or 10–25 mg/kg/min. -diethylamino-ethanol on the threshold dose of epinephrine required to produce ventricular tachycardia was measured in dogs anesthetized with cyclopropane. The administration of these agents produced a marked fall in arterial blood pressure and significantly raised the dose of epinephrine required to induce ventricular arrhythmias. If, however, the blood pressure was maintained at a relatively constant level by means of a pressure regulator inserted in the femoral artery the ability of these compounds to prevent epinephrine-cyclopropane arrhythmias was markedly reduced. When a pressure regulator was employed no significant protection could be demonstrated with -diethylaminoethanol. It is therefore possible that a considerable portion of the effect of procaine, procaine amide, and -diethylaminoethanol on ventricular arrhythmias is due to their hypotensive action.

Modification by sodium fluoroacetate (FA) of carotid sinus baroceptor responses to carotid occlusion. M. N. GURUSWAMI (introduced by M. B. CHENOWETH). *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

In dogs anesthetized with sodium pentothal and sodium barbital, the carotid sinuses were isolated on both sides. Toth vago-depressor nerves were cut. Blood pressure was recorded from a femoral artery. Initial blood pressure responses to bilateral carotid occlusion were obtained. Then both sinuses were, as far as possible, carefully isolated from the surrounding tissues and a 10% solution of FA (kept in plastic bottle) was applied locally. Recordings of the responses to carotid occlusion were taken at regular intervals. The carotids were ooccluded for definite periods each time. At first there was an apparent increase in the pressor response but between one and two hours later, varying with animals, it was found that the pressor response was depressed gradually, then abolished and finally, reversed. This type of reaction was not obtained after intravenous administration of even twice the LD_{100} doses of FA. Equimolar con-

centrations of sodium acetate or sodium fluoride did not affect the response. Freshly prepared solutions of FA failed to abolish the response but after combining with sodium fluoride they abolished and reversed the response. After keeping the freshly prepared solution of FA for a few days it reversed the pressor response. Only molar concentrations (10%) of FA elicited the reversal when applied locally. Glyceryl monoacetate given intramuscularly or applied locally, before or after FA, antagonizes the effects of FA. Local anesthetics, like procaine (2%), when applied similarly only depressed but did not reverse the pressor response. The significance of these reactions will be discussed.

Effects of potent analgesics upon vestibular function. LEONARD B. GUTNER,* WILBUR J. GOULD* AND ROBERT C. BATTERMAN. *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.*

The effects of potent analgesics upon vestibular function were studied because of the high incidence of dizziness, nausea and vomiting noted in ambulatory patients receiving these medications. The cold micro-caloric test and galvanic stimulation of the mastoid area were used in normal human subjects. The drugs studied were codeine sulfate 60 mg., meperidine hydrochloride 50 mg., morphine sulfate 10 mg. and methadon 10 mg. and all were administered parenterally. Five experiments were conducted with each drug. With the exception of codeine, the other analgesics produced increased labyrinthine sensitivity as evidenced by *1*) prolongation of the induced nystagmus, *2*) increase in the ratio of onset of nystagmus to its duration and *3*) decrease in milliamperage necessary to effect tilting. The results with codeine were indicative of a depression of vestibular response. The effect of meperidine, methadon and morphine upon vestibular function may contribute to the untoward reactions noted with the use of these drugs in ambulatory patients.

Relationship of blood and brain cholinesterase levels to acute oral toxicities of some organic phosphates. ERNEST C. HAGAN AND JOHN P. FRAWLEY (introduced by ARNOLD J. LEHMAN). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

The LD_{50}'s (mg/kg.) obtained for male and female rats, respectively, are for parathion (0,0-diethyl,0-p-nitrophenyl thiophosphate) 30 and 3; TEPP (tetraethylpyrophosphate) 2 and 1.2; E-838 (diethoxy thiophosphoric acid ester of 7-hydroxy-4-methyl coumarin) 42 and 19; OMPA (octamethyl pyrophosphoramide) 13.5 and 35.5; EPN (ethyl p-nitrophenyl thionobenzene phosphonate) 91 and 14.5; and DFP (diisopropyl-fluorophosphate) 13.5 and 7.7. Symptoms of poisoning for all of these phosphates are essentially the same. To correlate cholinesterase levels and severity of symptoms in acutely poisoned rats, LD_{75}'s of these phosphates were administered. This dosage was selected because it produced symptoms at a more convenient time than the LD_{50}. The first 5 animals from each group to show severe symptoms were sacrificed when in extremis and a corresponding number showing the mildest symptoms were sacrificed simultaneously. Brain, plasma, and red blood cell cholinesterase levels were determined in these animals. For each phosphate the average brain values, expressed as a percentage of the average of untreated controls, obtained in the 5 animals in extremis and in the 5 showing mildest symptoms were as follows: parathion 20% and 40%, TEPP 50% and 75%, E-838 25% and 45%, OMPA 90% and 90%, EPN 40% and 40%, and DFP 5% and 9%. Plasma and red blood cell cholinesterase levels ranged from 10–30% of the untreated controls, and showed no correlation with the severity of symptoms.

Failure of procaine amide (Pronestyl) to prevent cyclopropane-epinephrine induced ventricular tachycardia in dogs, or spontaneous arrhythmias occuring during cyclopropane anesthesia in humans. BRUNO HAID AND LUCIEN E. MORRIS (introduced by STUART C. CULLEN). *Div. of Anesthesiology, State Univ. of Iowa Hospitals, Iowa City.*

Procaine amide (Pronestyl) reportedly protects against epinephrine-induced ventricular tachycardia (VT) during cyclopropane anesthesia in dogs (MARK *et al. J.P.E.T.* 98: January, 1950), and is also recommended for the prevention and treatment of spontaneous ventricular irregularities during clinical anesthesia with cyclopropane (JOSEPH, S. I. AND E. A. ROVENSTINE. *Proc. Am. Soc. Pharmacol. & Exper. Therap.* Nov., 1950). Neither of these observations are supported by the study reported here. Eight animals were anesthetized with cyclopropane and, after equilibration, the individually standardized dose of epinephrine (ISD) which would regularly produce VT was determined for each dog. Pronestyl was then given intravenously in dosages of 10–60 mg/kg. body weight. During subsequently repeated injections of the ISD 7 of these 8 exhibited VT and 2 died of ventricular fibrillation. In 4 additional experiments 1 gm. of Pronestyl (60–140 mg/kg. body weight) was given intravenously as preanesthetic medication In the first animal when, after equilibration with cyclopropane, the ISD was injected, VT occurred and was immediately converted to ventricular fibrillation. Therefore, in the other 3 experiments the injection contained 5 gamma less epinephrine than the ISD. In all 3 VT occurred

which was of longer duration than had occurred after the ISD. Of 43 gynecological patients anesthetized with cyclopropane 24 were given Pronestyl either as premedication orally, or intravenously during anesthesia, or by a combination of both routes with a maximum dosage of 2.0 gm. Ventricular irregularities occurred in 14 of the 24 patients who received Pronestyl and VT was observed in 4. In the control group of 19 patients ventricular irregularities occurred in 8 and VT in 3. There was no significant difference in the incidence of ventricular irregularities in the two groups.

Comparison of capillary effects of ergotamine and the dihydrogenated ergot alkaloids. THOMAS J. HALEY AND MARGARET ANDEM.* *Atomic Energy Project, School of Medicine, Univ. of California at Los Angeles.*

A comparison of the capillary effects of ergotamine and the dihydrogenated ergot alkaloids has been made on the rat mesoappendix preparation using the method previously described (HALEY AND HARRIS. *J. Pharmacol. & Exper. Therap.* 95: 293; 1949). Topical application of these drugs showed that they produced vasoconstriction at doses ranging from 0.006–0.6 μg. The drugs would be rated in the following order: Dihydroergokryptine, ergotamine, dihydroergocornine, dihydroergotamine and dihydroergocristine. It is of interest that ergotamine is 100 times more potent than dihydroergotamine in this respect, whereas this latter compound and dihydroergocristine are equivalent. Both types of ergot alkaloids produce a definite modification of vasomotion but ergotamine has a more profound effect. The ergot alkaloids act more rapidly and have a more pronounced effect on the peripheral vascular bed than the β-haloalkylamines. However, tissue fixation resulting in an irreversible paralysis of the precapillary sphincters and decreased vasomotion is more pronounced with the β-haloalkylamines. Epinephrine reversal was observed with ergotamine, dihydroergocristine, dihydroergokryptine and dihydroergocornine but not with dihydroergotamine. Ergotamine and dihydroergokryptine were the most potent in this respect. Definite adrenergic blockade was observed at doses of 0.6–6 μg. Ergotmine was the most potent in this respect. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Reduction of anticholinesterase activity by anticholinergic agents. W. E. HAMBOURGER, HOMER B. FREESE,* DONALD L. COOK* AND D. M. GREEN. *Div. of Biological Research, G. D. Searle and Company, Chicago, Ill.*

The effect of anticholinergic compounds on the acute toxicity of di-isopropyl fluorophosphate (DFP) and tetraethyl pyrophosphate (TEPP) was studied in mice. These compounds included Banthine (β-diethylaminoethyl xanthene-9-carboxylate methobromide) and SC-3171 (β-diisopropylaminoethyl xanthene-9-carboxylate methobromide) both of which have neuro-effector (atropine-like) and ganglion blocking (TEA-like) activity; SC-1950 (2,6-dimethyl-1, 1-diethyl piperidinium bromide), a potent ganglion blocker; and atropine. All injections were intraperitoneal. Mortality curves for DFP or TEPP were determined in animals treated with a dose of one of the test compounds. The latter were given either 15 minutes before (prophylactic) or 3 minutes after (therapeutic) injection of the antiesterase. LD_{50} values were compared with those obtained from saline-treated controls. Prophylactically all compounds were effective against TEPP, and all except SC-3171 significantly reduced the toxicity of DFP. Therapeutically all compounds except SC-1950 significantly reduced the toxicity of DFP. SC-1950 alone was effective against TEPP.

Mechanisms of renal effects from morphine. CARROLL A. HANDLEY AND JOHN H. MOYER.* *Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

Morphine in a dosage of one or two mg/kg. has been demonstrated to powerfully depress several renal functions, including glomerular filtration, renal plasma flow and the ability of renal tubules to reabsorb glucose (Tm_G). In some experiments, morphine completely terminates these renal functions for periods up to 40 minutes. This phase is followed by a gradual return of activity, but renal functions may remain depressed as much as 50–60% for several hours. In studying the mechanism of these effects, the almost complete elimination of renal functions was found to be associated with a sharp reduction of blood pressure to about 50–60 mm. Hg, following the intravenous administration of the drug. The prolonged period, when renal functions were 50–60% of normal, was found to be associated with a mean pressure of 70–80 mm. Hg. These effects are almost certainly due to the precipitous changes in blood pressure since they may readily be duplicated by various other methods that produce similar depressions of blood pressure. Aside from the changes brought about by the blood pressure effect, morphine commonly depresses renal function by 20–40%, even if no change in blood pressure occurs, probably due to a redistribution of blood.

Comparison of several coronary vasodilators Khellin, papaverine, Aminophylline, epinephrine, nor-epinephrine, and Neo-Synephrine. HAROLD HARDMAN AND THEODORE R. SHERROD (intro-

duced by CARL C. PFEIFFER). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.*

This study was designed to make a comparative investigation of the coronary vasodilating properties of Khellin with some of the well established coronary vasodilators, as well as with some of the sympathomimetic amines. The method employed involved a direct cannulation of the coronary sinus in the barbitalized open-chest dog under positive artificial respiration with oxygen. A specially designed cannula was introduced into the coronary sinus through the right auricle and fixed securely by means of a purse string suture. Heparin was used as an anticoagulant and intravenous procaine was used to prevent ventricular arrhythmias. Coronary sinus outflow was led to the outside and measured periodically in a calibrated tipping bucket. The tipper was electrically wired so as to record the rate of flow kymographically. The blood was warmed and re-introduced into the femoral vein at such a rate as to maintain as nearly as possible a constant blood pressure. For Khellin, papaverine, and Aminophylline the average maximal response in coronary sinus outflow over a 3-minute period was compared with control levels. Intravenous doses of Khellin 5 mg/kg. produced an increase in coronary sinus outflow, averaging 98% greater than the control levels. Under identical conditions and with similar doses papaverine caused a 169% increase in coronary sinus outflow; while 20 mg/kg. doses of Aminophylline produced an average of 63% increase in outflow. In small doses (5–10 μg/kg.) all of the sympathomimetic amines studied caused a significant but transient rise in coronary sinus outflow.

Effect of botulinus toxins on biosynthesis of acetylcholine. WILLIAM D. HARKNESS, WALKER L. LOVING AND GEOFFREY WOODARD (introduced by BERT J. VOS). *Divs. of Pharmacology and Microbiology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Torda and Wolff (*J. Pharmacol. & Exper. Therap.* 89: 320, 1947) have suggested that inhibition of the synthesis of acetylcholine by botulinus toxins accounts for their mechanism of intoxication. Burgen *et al.* (*J. Physiol.* 109: 10, 1949) were unable to confirm this work. In view of these conflicting reports, the effect of botulinus toxin on another acetylcholine biosynthesizing system has been studied. Advantage has been taken of the observation of Dikshit, Feldberg, and others that the denervated small intestine synthesizes acetylcholine. Isolated rabbit intestinal strips were incubated in modified Van Dyke-Hastings Solution. At the end of 3 hours a demonstrable quantity of acetylcholine was found in the bathing fluid; after a second 3-hour period additional acetylcholine was present as determined by the frog rectus assay. The addition of type A botulinus toxin to the bath did not appreciably reduce the normal acetylcholine production. When the intestines of rabbits severely intoxicated following intravenous injection of type A toxin were employed a slight decrease in acetylcholine synthesis was observed occasionally in the first period but normal production resulted in the second 3-hour period. If, as has been suggested, the 'functional paralysis' observed in animals affected by Cl. botulinum toxins is due to decreased acetylcholine synthesis, the effect should be more profound than our results indicate.

Uptake of radioactive thyroxine by the tuber cinereum and hypophysis in thyroidectomized rabbits. EDWARD O. HARPER* AND PAUL A. MATTIS. *Dept. of Psychiatry and Pharmacology, Western Reserve Univ., Cleveland, Ohio.*

Thyroxine labeled with radioactive iodine, I^{131}, was injected intravenously into thyroidectomized virgin female rabbits. The average amount of radioactivity injected was 2.0×10^8 d/m. The concentration of I^{131} in tissue is expressed by the ratio, I^{131}/mg. organ wt/I^{131} mg. body wt. The rate of uptake of radioactive thyroxine by all parts of the brain, with the exception of the tuber cinereum, is much slower in the thyroidectomized rabbit. The maximum concentration in the brain is attained 12 hours following administration, whereas the tuber reaches a peak concentration 4 hours after injection. It is interesting to note that the behavior of the tuber cinereum toward radioactive thyroxine is essentially the same in both normal and throidectomized animals. The concentration of radioactivity by the pituitary of the thyroidectomized rabbit is greater than in the unoperated one, and the shape of its curve parallels that of the brain as a whole. Whereas the curves showing the rate of uptake of radioactive thryoxine by the pituitary and the tuber cinereum are almost identical in the normal animals, this relationship does not obtain in the thyroidectomized rabbits. Certain speculations on these differences will be discussed.

Deterioration of sodium monofluoroacetate in water and saline solutions. BERNICE L. HARRISON,* AURORA V. BRANSFORD* AND B. P. MCNAMARA. *Pharmacology Section, Med. Div., Army Chemical Center, Md.*

Toxicity experiments in our laboratories raised the question of the stability of water or saline solutions of sodium monofluoroacetate. Fourteen water solutions and 7 saline solutions were analyzed periodically over spans of 182 and 133 days, respectively. While both the water and saline solutions sustain an immediate loss of fluorine,

that in saline amounts on the average to about 4 times that in water. The water solution showed a significant progressive loss of F; the data are insufficient to distinguish between linear and exponential regression equations. The saline solutions showed no significant decreases in their contents of F after the initial loss. The behavior of solutions of fluoroacetate in urine is being studied also.

Adrenergic mechanisms in the chicken. STEWART C. HARVEY* AND MARK NICKERSON. *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

Previous reports of failure to obtain reversal of epinephrine vasopression with various adrenergic blocking agents and of complete inactivity of Dibenamine against epinephrine vasopression in chickens prompted studies to ascertain whether this species possesses adrenergic inhibitory mechanisms and β-haloalkylamine-susceptible pressor receptors. Urethane anesthesia was employed. Adrenergic inhibitory mechanisms are evidenced by vasodepression after Isuprel and by reversal of epinephrine-vasopression after 60 mg/kg. SKF-688A, N-benzyl-N-phenoxyisopropyl-β-chloroethylamine. Other β-haloalkylamines exhibit varying degrees of blocking ability at maximum tolerated doses. Dibenamine 140 mg/kg. completely abolishes but does not reverse the blood pressure response to epinephrine. Nor-epinephrine pressor responses and acetylcholine vasopression in atropinized chickens are blocked but not reversed by the above dose of SKF-688A. Ergotamine 1.5 mg/kg. causes vasoconstriction; it does not cause epinephrine reversal, but can combine with subeffective doses of β-haloalkylamines to cause reversal. Pressor responses to epinephrine are generally poor compared to those in mammals, although in chickens, epinephrine is more potent pressor agent than nor-epinephrine. Responses to angiotonin are variable and at times depressor. Responses to Pitressin are also variable. Histamine causes vasodepression. After death caused by 10 mg/kg. intravenous epinephrine, lung, liver and kidney were found to be markedly congested, the heart was dilated, and pronounced pleural effusion was noted; but the water content of the lung was increased only 13%. Dibenamine protects against these changes. We have confirmed other reports that Dibenamine, in doses inadequate to alter the pressor responses, protects chickens against death from epinephrine.

Central depression of the antihistaminics. MAX A. HEINRICH, JR. (introduced by CHARLES P. KRAATZ). *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

The effects of antihistaminic drugs on the CNS were studied by determination of synergisms with hexethal sodium and ether. The subcutaneous injection of 10 mg/kg. of the salt of an antihistaminic drug increased the 'sleeping time' of adult female rate receiving 80 mg/kg. of hexethal intraperitoneally 30 minutes later. Diphenhydramine was the most active closely followed in order by antazoline, Chlortrimeton, methapyrilene, thonzylamine and tripelennamine. Measured by this method, the CNS depression of diphenhydramine could be antagonized almost completely by 2 mg/kg. of amphetamine sulfate orally and slightly by daily pretreatment with 20 mg/kg. of diphenhydramine orally for three weeks. The abolition of the righting reflex in adult male mice exposed to 8% ether vapor in air was accelerated by the intraperitoneal administration of 10 mg/kg. of antihistaminic drug 10 minutes before the test. Diphenhydramine, Chlortrimeton and thonzylamine were moderately active, tripelennamine and antazoline only slightly. The spontaneous running of adult male rats in activity cages was unaffected by consumption in the diet of diphenhydramine 22.8, tripelennamine 21.6 and Chlortrimeton 10.6 mg/kg/day. Chlortrimeton ingested at a rate of 208.8 mg/kg/day caused a noticeable depression of spontaneous activity simultaneously with a slightly decreased ingestion of food.

Site of action of cation exchange resins in removal of sodium from the G. I. tract. ARTHUR E. HEMING AND THOMAS L. FLANAGAN (introduced by EDWIN J. FELLOWS). *Research Div., Smith, Kline and French Labs., Philadelphia, Pa.*

On the basis of indirect evidence it has been suggested that cation exchange resins bind sodium high in the G. I. tract and carry it out in the stool, thereby preventing the absorption of dietary sodium (MCCHESNEY AND MCAULIFF. *Am. J. Physiol.* 160: 264, 1905). In the present *in vitro* studies on capacity, Amberlite XE-96, a polycarboxylic cation exchange resin, was buffered at pH's from 2-8. It was found that the binding capacity was negligible below pH 4 and that it was probably insignificant in sodium removal below pH 5. Its increased capacity at higher pH's suggested that binding of dietary sodium might be very small. This assumption was confirmed by *in vivo* studies in dogs which demonstrated that this resin did not bind any significant amount of sodium in the stomach nor in the first 3 feet of the intestine. In fact, cations (potassium) bound to the resin when ingested were released by the acid in the stomach and not recombined in the intestine. This would indicate that the sodium which the carboxylic type resin carried out in the stool was removed from intestinal secretions rather than from dietary sources. Results of *in vitro* studies of the capacity of a sulfonic type resin

(Dowex 50) were consistent with the idea that binding of dietary sodium may occur in the stomach with this type of resin.

Effects of atropine on intestine of the dog before and after denervation. L. C. HENDERSHOT,* G. W. GRAY,* R. M. WHITROCK* AND M. H. SEEVERS. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Experiments reported previously (*Federation Proc.*, March, 1950) indicated that denervation of the intestine prolonged the inhibitory action of atropine. The following confirmatory observations have resulted from an extension of these studies. Before instituting the experiments described below, it was confirmed acutely that the lower bowel is stimulated markedly by electrical excitation of the pelvic and vagus nerves. Trained, unanesthetized animals with artificial openings into the terminal ileum, proximal colon, and distal colon were used. Intestinal motility was recorded on kymograph paper using the balloon-air-bromoform manometer method. The duration of inhibition of the intestine following an intravenous injection of atropine was studied before and after various denervation procedures. Parasympathetic denervation of the lower bowel does not modify significantly normal motility patterns but does change greatly the response to drugs. Transthoracic vagotomy or sacral parasympathectomy prolong the duration of atropine inhibition in the lower bowel by 27–72%, whereas both procedures in combination effect a prolongation of 118–141%. The prolongation of the atropine effect is greatest in the distal colon following sacral parasympathectomy, while vagotomy produces its greatest effect in the terminal ileum. One month after lumbar cord section the duration of atropine inhibition of the colon is decreased. Section of the aortic plexus appears to produce effects comparable to vagotomy.

Pharmacologic studies with (2-Phenyl-2-chloro) ethylmethylbenzylamine in animals and man. F. G. HENDERSON, D. PIETZ,* K. G. KOHLSTAEDT* AND K. K. CHEN. *Lilly Research Labs., Indianapolis, Ind.*

A series of 2-Aryl-2-chlorethyl dialkylamines were studied for their adrenergic-blocking effect. Each compound investigated showed some activity when administered by vein. One of this series, (2-Phenyl-2-chloro) ethylmethylbenzylamine hydrochloride (compound 08353), was a potent epinephrine-reversal agent by the intravenous route and was also effective by mouth when administered to dogs. In cats an intravenous dose of 1.0 mg/kg. reversed the pressor response to 2.5 μg/kg. of epinephrine hydrochloride. Orally, doses of 200–300 mg. 3 times a day reversed the pressor response to epinephrine in the dog. Since this compound was tolerated orally by animals, preliminary studies were carried out in 8 human subjects. Compound 08353 was given in doses of 200–300 mg. after each meal. Maximum doses produced some nausea and vomiting. In 6 subjects blood pressure studies were made following intravenous injection of epinephrine hydrochloride. Five of the 6 subjects showed a decrease in the pressor response to epinephrine hydrochloride following oral administration of compound 08353.

Effect of vasomotor drugs on flicker fusion frequency. CHARLES D. HENDLEY (introduced by DONALD SLAUGHTER). *Dept. of Physiology and Pharmacology, Univ. of South Dakota, Vermillion.*

Krasno and Ivy (*Circulation* 1: 1267, 1950) have used the effect of nitroglycerin on the flicker fusion frequency (c.f.f.) as a diagnostic aid in cardiovascular disorders. They found that nitroglycerin lowered the c.f.f. in normals, but raised it in persons with vasospastic tendencies. The present study was undertaken to elucidate the mechanism of these changes. The Krasno-Ivy flicker 'photometer' was used. A drop in c.f.f. of about 1.6 cycles/second was found in normal young males following administration of 0.43 or 0.65 mg. of nitroglycerin. 0.32 mg. gave a somewhat smaller drop, and 0.16 mg. had no effect. Placing the subject in a recumbent position did not alter the result, indicating that the change in c.f.f. is not due simply to the effect of gravity on retinal blood supply. Vasopressin (5–12 pressor U, intramuscularly) also lowered the c.f.f. by 2 cycles/second or more. Epinephrine (0.3 and 0.4 mg., intramuscularly) lowered the c.f.f. by a mean of 1.6 cycles/second. Changes in blood pressure with both of these drugs were minimal. When small doses of nitroglycerin (0.16 or 0.32 mg.) were given while the c.f.f. was lowered by vasopressin or epinephrine, the c.f.f. promptly rose to normal in 2–6 minutes whereas recovery took one-half to 1 hour if the vasodilator were not given. The results of all these observations are consistent with the view that small vasomotor changes in the retina, either in the direction of dilation or of contriction, cause a small but measurable impairment in visual function as measured by the c.f.f.

Barbiturate antagonism of antihistaminic overdosage. WOODROW C. HERBERT* AND E. LEONG WAY. *Div. of Pharmacology and Experimental Therapeutics, School of Medicine, and College of Pharmacy, Univ. of California, San Francisco.*

Inasmuch as the toxic effects of the antihistaminics are convulsive in nature, it was felt prudent to determine whether or not a central nervous system depressant would antagonize these actions.

White mice of both sexes weighing from 15–25 gm. were used as laboratory animals. Intraperitoneal LD_{50}'s were determined on 4 antihistaminic drugs: diphenhydramine (Benadryl), phenindamine (Thephorin), tripelennamine (Pyribenzamine), and chlorprophenpyridamine (Chlor-trimeton). These results were compared with LD_{50}'s obtained on the same drugs after having previously injected the animals with 30 mg/kg. of sodium pentobarbital intraperitoneally. The LD_{50}'s of 3 of the 4 drugs tested were increased 1.4–1.8 times by the use of the barbiturate. Sodium pentobarbital had no effects upon the LD_{50} of chlorprophenpyridamine (Chlor-trimeton); however, the onset of death of the animals was appreciably delayed. Observation of the animals during the experiment indicated that the convulsions resulting from the administration of antihistaminics, while not completely aborted, were less severe when preceded by the barbiturate.

Curare compounds. IX. Curariform activity of Calabash-toxiferin I chloride. D. A. HERRING* AND DAVID F. MARSH. *Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgantown.*

Schmid and Karrer have isolated several alkaloids from gourd curare of the Middle Orinoco region. Little investigation of their pharmacological activity has been made. We have investigated the activity of a sample of Calabash-toxiferin I chloride, $C_{20}H_{22}ON_2Cl$, generously provided by P. Karrer of Zurich. This material is similar or identical with Toxiferin isolated from Strychnos toxifera bark by H. Wieland. Head drop lasting 3–7 minutes occurs in rabbits that receive 5.1–5.5 μg/kg. (1.4×10^{-8} M/kg.) intravenously. The cross-over doses of *d*-tubocurarine chloride and *d*-O-methyltubocurarine iodide to produce similar effects in these same rabbits are 145–155 μg. and 16–18 μg/kg., respectively. Complete paralysis of the respiratory muscles occurs with doses of 7–8 μg/kg. Some preliminary respiratory stimulation was observed but there were no other obvious side effects with the head drop doses. Intraperitoneal injection of 11 μg/kg. in white mice produces inability to cling on an inclined screen in 60% of the animals. The LD_{50} is 15–17 μg/kg. Doses of 15 μg/kg. in the barbitalized cat which produce abolition of gastrocnemius muscle response do not markedly change the blood pressure or pulse rate. Head drop occurs in dogs that receive 4.9–5.4 μg/kg. intravenously. The onset is slower and the effects are more prolonged than with *d*-tubocurarine. There is no nausea, vomiting, or apparent loss of consciousness. On either a molecular basis or a weight basis, this material is apparently the most potent naturally occurring curariform agent known.

Effects of anxiety and morphine on discrimination of painful stimuli. H. E. HILL,* C. H. KORNETSKY,* H. G. FLANARY* AND ABRAHAM WIKLER. *Natl. Inst. of Mental Health, Natl. Insts. of Health, Research Branch, U. S. Public Health Service Hospital, Lexington, Ky.*

The effects of subcutaneous injection of 15 mg. of morphine on the ability of subjects (post-addicts) to estimate intensity of pain were studied under two conditions: *a*) proceeding with the experiment without familiarizing the subjects with the potentially fear-inspiring experimental situation; *b*) preceding experimentation with reassurance, demonstrations and explanations designed to allay apprehension and also permitting the subject to administer the stimuli to himself. Under all conditions, 15 electric shock stimuli of standard strength, followed one hour later by 6 successive series of stimuli of variable strength were delivered to one hand of each subject, who was required to state whether the shocks were stronger or weaker than the standard. Under *condition a* the subjects tended to overestimate the intensity of applied shocks, and this error was reduced by morphine. Under *condition b* overestimation of the intensity of applied shocks was less, and morphine had no significant effect thereon. Tentative conclusions are drawn that *1*) anxiety sensitizes the organism to stimuli that have particular values; *2*) morphine does not materially alter the differential perception threshold for electric shock stimuli when anxiety has been dissipated; *3*) morphine reduces anxiety related to pain; *4*) the possible influence of anxiety upon sensory thresholds should be taken into account in investigations of this type. The validity of these conclusions is being tested by further experimentation.

Influence of low oxygen tension and lowered glucose levels on uterine activity. JAMES G. HILTON* AND ROBERT A. WOODBURY. *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

These studies on the isolated rabbit uterus were conducted after the animal had received 10 mg. of diethylstilbestrol daily for 7 days. Each uterine horn was divided into 4 equal portions and each portion was suspended in Tyrodes solution while the oxygen and glucose contents were varied. A reduced response to oxytocic agents was observed with low oxygen and low glucose levels. This reduction was more marked in the environment of low glucose. Incomplete relaxation also occurred in the low glucose environment. The relationship of these observations to the high uterine tone present in patients with dystocia dystrophy will be discussed. (Supported by a U. S. Public Health Service Research Grant.)

Depression of transmission through sympathetic ganglia produced by inhalation anesthetic agents. DUNCAN A. HOLADAY AND MARTIN G. LARRABEE (introduced by E. M. PAPPER). *Dept. of Anesthesia, Columbia-Presbyterian Med. Center, New York City, and Dept. of Biophysics, Johns Hopkins Univ. and Hosp., Baltimore, Md.*

The effect of the various planes of surgical anesthesia, as produced by di-ethyl ether and chloroform, on transmission through sympathetic ganglia has been tested in the cat. Condenser discharge shocks were delivered to the distal stumps of severed preganglionic fibers, and the postganglionic action potentials from the *in situ* superior cervical ganglion were amplified and projected onto a cathode ray oscilloscope. Responses to maximal preganglionic volleys at frequencies greater than 18 per second were depressed roughly proportional to the depth of anesthesia in every case. Responses to single maximal volleys and infrequently repeated volleys were depressed significantly in 3 out of 5 animals tested. Studies with graded strengths of stimuli revealed different groups of fibers to have different sensitivities to blockade by the anesthetic agents. Ether and chloroform seemed to be equally effective in producing blockade. Preliminary tests suggested that the barbiturates were less effective.

Immunological studies on a highly purified cholinesterase. W. C. HOLLAND (introduced by MARGARET E. GREIG). *Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

We have previously found that by inhibiting cholinesterase activity of erythrocytes by certain drugs, one can produce an increase in hemolysis. We therefore decided to prepare a specific antibody for cholinesterase and study its effects on hemolysis and cholinesterase activity. Seven male rabbits were immunized with a highly purified preparation of beef erythrocyte cholinesterase obtained from Winthrop-Stearns, Inc., the total dose being between 30 and 50 mg. The serum of rabbits immunized with the enzyme preparation was found to contain a relatively high titre of immune hemolysin (1-500—1-800). This serum, when fresh, also inhibited cholinesterase by 40-60%. The heated antiserum (56° for 30 minutes) showed much less inhibitory activity of cholinesterase and caused less hemolysis of beef erythrocytes. Both the hemolytic activity and the cholinesterase inhibitory effect of the heated serum could be restored by addition of fresh guinea pig serum. Under our experimental conditions there was a close correlation between hemolysis of beef erythrocytes and inhibition of cholinesterase activity by the immune serum. The greater the degree of cholinesterase inhibition the greater the hemolysis. The correlation between hemolysis and cholinesterase inhibitory action of serum resembled the similar correlation of these effects found with physostigmine. Experiments reported suggest that in the case of beef erythrocytes cholinesterase may be the antigen present in red cell stroma which stimulates the production of hemolysins when rabbits are immunized w th stroma or fractions thereof.

Aryl triiodo alkanoic acid derivatives as cholecystographic media. JAMES O. HOPPE AND S. ARCHER.* *Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

Soft tissue structures such as the gallbladder are not visible on the ordinary roentgenogram. The gallbladder can be visualized by means of radiopaque substances which upon oral administration are absorbed and selectively excreted into the bile. The cholecystographic properties of a series of α-(3-amino-2,4,6-triiodobenzyl)-alkanoic acid derivatives were studied by means of a test procedure using cats. Comparative data were obtained on iodoalphionic acid and certain related diiodo compounds. It was observed that optimum visualization of the cat gallbladder was obtained when the number of carbon atoms in the alkanoic acid side chain approached five, whereas with the diiodo series, peak visualization occurred as the number of carbon atoms in the side chain approached seven. The most effective compound in this series in respect to gallbladder visualization in cats and acute oral toxicity in mice was 3-(3-amino-2,4,6-triiodophenyl)-2-ethylpropanoic acid (WIN 2011). In comparison with iodoalphionic acid upon oral administration to cats at a dose of 100 mg/kg., WIN 2011 was found to be 1.4 times as effective in visualizing the gallbladder. The acute oral LD_{50} for WIN 2011 in mice was 16 gm/kg. compared with 3.8 gm/kg. for iodoalphionic acid. Repeated oral doses of 2 mg/kg. of WIN 2011 were tolerated without evidence of gastrointestinal irritation or impairment of liver or kidney function in dogs.

Oxygen removal, oxygen consumption and ventilation in normal and cardiac male subjects, during the basal state and the first minute recovery from a standard exercise. JOHN B. JOHNSON,* EDWARD H. HALE* AND EUGENE CLARKE* (introduced by A. H. MALONEY). *Cardio-Vascular Research Lab. of Howard Univ. College of Medicine, and Freedmen's Hospital, Washington, D. C.*

A study of oxygen removal, oxygen consumption and ventilation per minute/B.S.A.M.[2] was made in normal and cardiac subjects in the basal state and during each of the first 4 minutes following exercise. The exercise consisted of having the subject stand from the sitting position 20 times in 1

minute. Expired air was analyzed in duplicate using a Scholander gas analyzer. Normal and cardiac subjects were evaluated clinically by electrocardiogram, cardiac surface area and vital capacity. The basal oxygen consumption in normal subjects was 123 ± 12 cc. and 417 cc. during the first minute recovery. In cardiac subjects, the mean oxygen consumption in the basal period was 142 cc. but was the same as the normals in the first minute recovery period (412 cc.). Hyperventilation was present in cardiac subjects during both the basal and recovery periods. The basal ventilation in normal subjects was 2.81 ± 0.5 liters, first minute recovery 7.42 ± 1.29 liters. The mean ventilation for cardiacs in corresponding periods was 5.70 liters and 10.72 liters, respectively. The basal oxygen removal in normal subjects expressed in cc. per liter ventilation was 44.3 ± 4 and 56.9 ± 6 during the first minute recovery. The corresponding mean values in cardiacs was 26.0 cc. or 59% of normal and 38.9 or 68% normal. This study appears to provide an accurate technique in assessing cardiac insufficiency.

Acetylation of sulfanilamide in fluoroacetate-poisoned rats. LINARES B. JOHNSON* AND MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Sprague-Dawley rats were injected intraperitoneally with a total of 225 mg/kg. of sulfanilamide in 3 equally divided doses at hourly intervals. Urine was collected for 23 hours from groups of 3 rats each. The urinary excretion of free sulfanilamide and total sulfanilamide after acid hydrolysis was determined by the method of Bratton and Marshall (*J. Biol. Chem.* 128: 537, 1939). The difference was taken to be the amount of acetylated sulfanilamide excreted. Rats injected with sodium fluoroacetate (1 mg/kg., an approximate LD_5) received the same sulfanilamide treatment beginning 1 hour after the administration of the poison. Acetylation of sulfanilamide was increased by 13% ($P = .01$) in animals receiving fluoroacetate; no significant difference in total sulfanilamide excretion between the control and poisoned animals was noted. At larger doses of fluoroacetate the higher mortality and the anuria in surviving animals prevented the determination of acetylation rate. Comparison of other mono-fluoro-acids and their effects upon acetylation in this and other species will be reported. In addition, the effect of acetyl-precursors on these phenomena will be discussed.

Diurnal variations of renal function in cirrhosis. RICHARD A. JONES,* GERALD O. MCDONALD,* E. E. BOND* AND JULES H. LAST. *Dept. of Experimental Medicine, Northwestern Univ. Med. School and Veterans Administration Hosp., Hines, Ill.*

Diurnal rhythm in renal excretion of water and electrolytes has been previously noted by many investigators. Quantitative renal function tests were made in a series of 6 cases of cirrhosis with ascites to determine the pattern of diurnal rhythm in this type of peripheral edema. Concurrently with a 24-hour clearance period, short periods were run between 8–12 A.M., and compared with those run between 12–4 A.M. at night. The patients studied in this small series showed a reversal of normal diurnal rhythm in that nocturnal inulin clearances (GFR) were consistently greater than daytime values (mean diff = 20.3 cc/minute, $P < 0.01$). The reversal in diurnal GFR pattern in this series of cirrhotics is similar to that reported in cardiacs. GFR values were within normal limits. In contrast to cardiacs, cirrhotics demonstrated a decrease in nocturnal urine flow and a corresponding increase in inulin U/P ratios. Cirrhotics were thus able to concentrate their nocturnal urine flow in a normal manner, despite the increase in filtration rate. The rise in nocturnal inulin U/P ratios in the face of increases in filtration rates would speak for an increase in distal tubular resorption of water during sleep. Inulin U/P ratios in cirrhotics (81.3 ± 12.8) were not significantly higher than those obtained in normal men. The data also emphasized the need to study renal function over 24-hour periods. Twenty-four-hour inulin U/P ratios were significantly higher than similar U/P ratios obtained from either day or night spot periods of 20–30 minutes duration. Twenty-four-hour GFR values corresponded more closely to those obtained during nocturnal spot periods than those run during the day.

A metabolite of pamaquine from chickens. EDWARD S. JOSEPHSON, JOSEPH GREENBERG, D. JANE TAYLOR AND H. L. BAMI (introduced by N. B. EDDY). *Natl. Insts. of Health, Natl. Microbiological Inst., Bethesda, Md. and Malaria Inst. of India, Delhi.*

A 5,6-quinolinequinone has been isolated from chickens following pamaquine administration. The crystalline dipicrate salt of the quinoxaline derivative of this metabolite has been obtained through interaction of this quinone with *o*-phenylenediamine. A single oral dose of pamaquine naphthoate, equivalent to 6 mg. base/kg. body weight, was administered to chickens. Droppings, collected over 24 hours, were adjusted to pH 8.9 with sodium acetate and the metabolite was extracted with ethylene dichloride. The quinone was then concentrated into a small volume of 1 *N* HCl and treated for 24 hours with *o*-phenylenediamine to form a quinoxaline. The quinoxaline was partially purified by chromatography on a cellulose column. A minute quantity of a dipicrate salt of this quinoxaline was obtained (m.p. 190–195° C.), which is

being further purified. 8-(diethylamino-1-methylbutylamino)-5,6-quinolinequinone (I) was converted into a crystalline quinoxaline dipicrate (m.p. 202° C.) by Drs. R. E. Elderfield and E. Werble of Columbia University. Ultraviolet absorption spectra, antimalarial activity *in vitro* and methemoglobin producing properties of our quinolinequinone are almost identical with those of the synthetic product I. Physical properties of our quinoxaline dipicrate also suggest that one of the metabolic products of pamaquine is a quinolinequinone of type I.

Identity of procainesterase and pseudo-cholinesterase. WERNER KALOW (introduced by CARL F. SCHMIDT). *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*

Ultraviolet spectrophotometry was applied as a means of studying the hydrolysis of procaine and benzoylcholine by human serum esterase. Competition of both substrates for one esterase could be directly demonstrated by observing separately their fate at 300 and 235 mμ. Both are competitive inhibitors for each other as also revealed by standard mathematical analysis of the hydrolysis curves. Inhibition by procaine of the hydrolysis of acetylcholine by serum esterase is already known. A corresponding competitive inhibition of procaine hydrolysis by means of acetylcholine is demonstrated in this study. Purified pseudo-cholinesterase (Harvard serum fraction IV-6-3, Cohn and co-workers) also attacks procaine, and all relations concerning reaction rates and competition between the different substrates are the same as with crude esterase preparations. During procaine hydrolysis, one molecule of procaine is attached to an enzyme center per unit of time. The dissociation constant (Michaelis constant) for this enzyme-substrate complex is $5.5 \cdot 10^{-6}$M at pH 7.4 and 32 C. If a second procaine molecule enters this complex ($K_2 = 2.2 \cdot 10^{-4}$M), the esterase activity is inhibited. The interaction between procaine and pseudo-cholinesterase is of neuro-physiological importance, since this enzyme has been demonstrated in cells of the sensory nervous system (Koelle). The identity of serum 'procainesterase' and 'pseudo-cholinesterase' may facilitate the study of this enzyme in liver diseases due to the simplicity of direct spectrophotometry with procaine as a substrate. The method can be adapted to studies on many aromatic esters.

Relation of mono-fluoro fatty acids to citrate levels in intact animals. ALEXANDER KANDEL,* LINARES B. JOHNSON* AND MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Experiments with dogs, rabbits and rats indicate that accumulation of citrate may be considered an index of poisoning, but not necessarily the cause of death following administration of omega-monofluoro fatty acids. Increased citrate levels in brain may or may not be accompanied by convulsive death in either rats or dogs. On the other hand, convulsions, particularly those induced by methyl 4-fluorobutyrate, occur at brain citrate levels which are in the range of control levels. Essentially normal brain citrate levels were obtained in dogs partially protected by the induction of anemic anoxia with *p*-aminoacetophenone prior to the administration of the fluoro compounds, yet convulsions have occurred. Rabbits treated with fluoroacetate have high citrate concentrations in the heart muscle, and poisoning terminates in ventricular fibrillation. Those treated with methyl 4-fluorobutyrate have as high citrate levels in heart muscle as those poisoned with fluoroacetate, although death appears to result from cardiac failure, not fibrillation. Rats show little correlation between citrate accumulation in brain and toxic manifestations resulting from the administration of the fluoro compounds. It is probable that the factors responsible for the accumulation of citrate in fluoro-fatty acid poisoned animals are not the identical factors responsible for production of symptoms of poisoning and death.

Effects of parpanit on sympathetic ganglia. ALEXANDER G. KARCZMAR,* DONALD F. BOGDANSKI* AND THEODORE KOPPANYI. *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

The ganglionic depressant effects of parpanit HCl were tested in nembutalized atropinized dogs. Under these conditions 10 mg/kg. of parpanit produced, on slow i.v. administration, a temporary fall of blood pressure and a more prolonged cardiac slowing not reversed by even 30 mg/kg. of atropine. Larger doses caused prolonged hypotension but only toxic doses (30–40 mg/kg.) had effect on respiration. The ganglionic activity was tested prior to administration of parpanit, by recording pressor responses to acetylcholine, nicotine and to stimulation of the cervical sympathetic. 10 mg/kg. of parpanit converted acetylcholine pressor effects into depressor responses (cf. KARCZMAR AND KOPPANYI. *J. Pharmacol. Exper. & Therap.* 98, 17, 1950) and partially inhibited pressor effects of cervical sympathetic and of nicotine for about 20 minutes. A complete and somewhat more prolonged ganglionic block resulted from larger doses. Physostigmine and TEPP deblocked only to a very limited extent the partially or completly inhibited ganglia. In all cases physostigmine potentiated the cardiac slowing by parpanit and caused respiratory depression. Death resulted in eserinized (2–5 mg/kg.) animals with as little as 10 mg/kg. of parpanit and could not be prevented

by artificial respiration. Ganglionic depressant effects of parpanit are thus characterized by their short duration and differ from those of other ganglionic blocking agents in being little antagonized by anticholinesterases.

Respiratory effects of some new analgesics. ARTHUR S. KEATS,* AND HENRY K. BEECHER. *Anesthesia Lab. of Harvard Med. School at Massachusetts General Hosp., Boston.*

Each of 15 normal men received the following drugs: saline (1.0 cc.), sodium pentobarbital (200 mg/70 kg. intramuscularly), morphine sulfate (10 mg/70 kg.), 1-iso-methadone (10 mg/70 kg.), *dl*-4,4-diphenyl-6-morpholinoheptanone-3-hydrochloride (Heptazone) (60 mg/70 kg.), 6-methyldihydromorphine (30 mg/70 kg.), methyl-dihydromorphinone hydrochloride (Metopon) (3 mg/70 kg.). Each agent was administered subcutaneously on separate occasions. Except for saline and pentobarbital, these doses had been previously determined to be equivalent analgesic doses. The following measurements were made: resting minute volume and respiratory rate, minute volume and rate response to inhalation of 5% carbon dioxide in oxygen. This dose of pentobarbital depressed respiratory function only slightly when compared with saline. Morphine significantly depressed all functions measured except the rate response to carbon dioxide. The depression from the other 4 narcotics was similar in degree to morphine. No differences in any measurement were revealed by analysis of variance of the data of the 5 morphine-like drugs. The average values (in %) for pentobarbital and morphine, respectively, are (100 is the effect of saline): resting minute volume 98.1 and 87.2, resting respiratory rate 98.1 and 87.7, minute volume response to carbon dioxide 104.6 and 85.5, rate response to carbon dioxide 99.2 and 94.7. The question of an inherent relationship between analgesic action and respiratory depression will be discussed.

Tetraethylammonium ion and cholinesterase activity. C. J. KENSLER AND R. W. ELSNER.* *Dept. of Pharmacology, Cornell Univ., New York City.*

Several of the pharmacological actions of large amounts of tetraethylammonium ion (TEA) would be explainable if TEA possessed a weak anticholinesterase activity. However, Barlow and Ing (*Brit. J. Pharmacol.* 3: 298, 1948) did not observe any inhibition of horse serum cholinesterase by TEA in a concentration of 0.0003 M. In the present study the effect of TEA on cholinesterase activity from different sources and with various substrate concentrations was examined. TEA in concentration of 0.001 M and higher was found to inhibit human serum and rat brain cholinesterase activity when low substrate concentrations (ca. 0.001 M) of acetylcholine were employed. With high substrate concentrations (0.015 M), serum cholinesterase activity was not significantly inhibited and brain cholinesterase activity was increased. A study of the substrate concentration-activity relationship of serum cholinesterase in the presence of TEA (0.006 M) proved that the TEA inhibition was a competitive one. A study of the substrate concentration-activity relationship of brain cholinesterase showed that TEA (0.006 M) inhibited activity with substrate concentrations up to, and slightly higher, than that giving optimal velocity (0.0025 M) and increased activity at higher substrate concentrations. A similar pattern was found using human erythrocyte cholinesterase. The accelerating action of TEA is attributed to a competition between TEA and acetylcholine for the centers of the enzyme with which excess acetylcholine combines to produce the phenomena known as substrate inhibition. Experiments with hexamethonium ion, also a weak inhibitor of cholinesterase activity, have yielded similar results.

Sodium concentration and action of cocaine and *d*-tubocurarine on rat diaphragm preparation. C. J. KENSLER AND DONALD NUSSBAUMER.* *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

Lowering the sodium concentration of Tyrode's solution with replacement by sucrose has been observed (KENSLER AND VAUGHN WILLIAMS. *J. Pharmacol. & Exper. Therap.* 101: 1951) to render the rat diaphragm preparation, stimulated through its nerve, more susceptible to the blocking action of compounds possessing local anesthetic properties. In the present study, low sodium concentrations were found to increase the blocking action of cocaine and *d*-tubocurarine chloride (DTC). Attempts to establish a quantitative relationship for this antagonism between sodium and the blocking action of cocaine, procaine or DTC were not successful due to the fact that the action of these compounds was not readily reversible in the low sodium solutions. DTC is also antagonized in this preparation by potassium. (QUILLIAM AND TAYLOR. *Nature* 160: 603, 1947) and by calcium (KENSLER. *Federation Proc.* 8: 307, 1949). The blocking action of amylocaine is increased in potassium free solutions (PECZENEK AND WEST. *Nature* 164: 354, 1949). As FATT AND KATZ (*J. Physiol.* 111: 46, 1950) have presented evidence indicating that the amount of acetylcholine released at nerve endings is reduced in low sodium solutions and as Harvey found a similar failure in low calcium solutions, decreased acetylcholine liberation is probably an important factor in the increased sensitivity when sodium or calcium is low. Potassium low solutions were found to increase the concentration (from 0.024–

0.033 M) of sodium required for a minimal response of the rat diaphragm to nerve stimulation.

Experimental tolerance to hyperglycemia produced in rabbits by analgesic compounds. K. K. KIMURA, B. DEBOER, L. WALTS* AND E. KEITH.* *Dept. of Pharmacology, Saint Louis Univ. School of Medicine, Saint Louis, Mo.*

It was shown in a previous report (Second Fall Meeting, A.S.P. & E.T., Boston, Nov. 13–15, 1950) that intravenous injections of Nisentil, Dromoran, Morphine and Methadone produce significant hyperglycemia with peak increases of 50–77% over contral levels. The present report is on the production of tolerance to the hyperglycemic responses produced by the above analgesics. All drugs were injected in the marginal ear veins of albino rabbits daily for 9 weeks with the following dosages (mg/kg.): Nisentil 5, Dromoran 2, Morphine 5, and Methadone 2. Control injections were made with normal saline solution and barbital sodium 150 mg/kg. or pentobarbital sodium 20 mg/kg. Blood sugar determinations on venous blood were made weekly using the Somogyi-Nelson colorimetric method. Blood samples were taken before, and ½, 1, 3, and 6 hours after the injections of drugs. Saline produced no significant blood sugar changes. Hyperglycemic responses to the first injection of analgesic compounds were used as basal levels of hyperglycemia. Subsequent weekly blood sugar determinations indicated production of tolerance to the respective drugs in the order of activity: Morphine > Dromoran > Methadone > Nisentil. The significance of the results of the experiment will be discussed.

Factors in epinephrine 'reversal.' THEODORE O. KING (introduced by T. KOPPANYI). *Univ. of Wyoming College of Pharmacy, Laramie.*

In earlier experiments (T. O. KING AND T. KOPPANYI. *J. Am. Pharm. Assoc.*) it was demonstrated that ergotamine tartrate (0.05 mg/kg.) was capable of reversing the effects of various adrenergic blocking agents. Subsequent experiments have shown that this 'anti-adrenolytic' effect of ergotamine can be modified in several ways: *1*) By administration of supra-blocking doses of the adrenolytic agent. *2*) By administration of ephedrine sulfate (1–2 mg/kg.). *3*) By administration of cocaine hydrochloride (10 mg/kg.). *1*), *2*), and *3* will each reduce or completely block the effects of ergotamine in reversing dibenzamine blockade of epinephrine pressor effects. In addition, they will prevent ergotamine reversal of the depressor effects of N-isopropylarternol hydrochloride. The close pharmacological interrelationship of cocaine, ergotamine, and ephedrine have been noted with respect to the following phenomena: *1*) Small doses of all three compounds sensitize the pressor effects of epinephrine and arterenol. *2*) Cocaine and ephedrine sensitize the vasodepressor effects of N-isopropylarterenol. *3*) Ergotamine tartrate (0.05 mg/kg.) reverses the vasodepressor effects of N-isopropylarterenol. Combined administration of cocaine hydrochloride (10 mg/kg.) and ephedrine sulfate (2.0 mg/kg.) also often reverse these effects. *4*) Ephedrine and cocaine are independently capable of antagonizing the anti-adrenolytic effects of ergotamine.

Pharmacological effects of isoquinolinium compounds. THEODORE O. KING (introduced by T. KOPPANYI). *Univ. of Wyoming College of Pharmacy, Laramie.*

Pentamethylenebis (isoquinolinium bromide), decamethylenebis (isoquinolinium bromide), and *p*-xylylenebis (isoquinolinium bromide) were tested for their effectiveness in antagonizing the Straub phenomenon on the tails of morphinized mice. Only the *p*-xylylene derivative was effective. Intraperitoneal injection of 50 gamma of this compound completely abolished the tail reaction and the characteristic excitement produced by 0.5 mg. of morphine sulfate in 20-gm. mice. Relaxation of the tails occurred from 3–7 minutes following administration of the *p*-xylylene compound, and was comparable to the same effects produced by intraperitoneal injection of 3 gamma of *d*-tubocurarine chloride pentahydrate. The pentamethylene and decamethylene compounds were ineffective in combatting the morphine effects. Head drop in rabbits was produced by intravenous injection of 1.5 mg/kg. of the *p*-xylylene compound but could not be effected by doses as high as 20 mg/kg. of the other two analogs. The margin of safety between the curarizing dose and the lethal dose is very narrow. Death occurred in rabbits within 15 seconds following intravenous administration of 2.0 mg/kg. The collapse of the animal appears to be nicotine-like in character rather than typically curariform. The LD_{50} in mice is approximately 5 mg/kg. A lethal dose could be successfully antagonized in both rabbits and mice by protection with 0.2 mg/kg. neostigmine sulfate administered immediately before or immediately after injection of the *p*-xylylene compound. The pentamethylene derivative is capable of potentiating the nicotinic rise in blood pressure produced by acetylcholine in atropinized nembutalized dogs. When administered to mice poisoned by the *p*-xylylene analog (5 mg/kg.) the pentamethylene compound (5 mg/kg.) appeared to accelerate the lethal effect and effected no protection.

Effect of nitrites and nitrates on oxygen uptake of arterial tissue. M. JOYCE KNAPP,* C. JELLEFF CARR AND JOHN C. KRANTZ, JR. *Dept. of Phar-*

macology, School of Medicine, Univ. of Maryland, Baltimore.

The effect of nitrites and organic nitrates upon the respiration of the thoracic aorta of the rat has been studied to determine whether alteration of this phase of metabolism of smooth muscle is responsible for the vasodilating properties of these drugs. It has been previously shown that the organic nitrites and nitrates, acutely-acting drugs, interfere with the ATPase activity of arterial tissue, whereas sodium nitrite does not (KRANTZ *et al.*, 1950). Significant inhibition of oxygen consumption by sodium nitrite was found in this study, however, whereas sodium nitrate produced no apparent effect. The organic nitrites, octyl and amyl nitrite and the nitrates, glyceryl trinitrate and erythritol tetranitrate, did not significantly interfere with the oxygen uptake of arterial tissue. The relationship between ATPase inhibition, oxygen uptake and vasodilatation produced by the drugs of the nitrite-nitrate series is discussed.

Adrenochrome redox system. GEORGE B. KOELLE AND JONAS S. FRIEDENWALD. *Wilmer Inst., Johns Hopkins Univ. and Hosp., Baltimore, Md.*

Recent studies by J. Harley-Mason (*J. Chem. Soc.* p. 1276, 1950) of the reduction products of adrenochrome have been interpreted as indicating that in 0.275 M solution the compound exists as a zwitterion *p*-quinoneimine, which on reduction to leukoadrenochrome takes up only one atom of hydrogen per molecule and undergoes irreversible dismutation. We have studied polarographically the reduction of 0.001 M solutions of pH 6.0–8.0 at 3°C. by hydrogen-platinized asbestos, and have measured the hydrogen-uptake in the Warburg apparatus at the same concentration and pH range, using either platinized asbestos or palladinized barium sulphate as catalyst. Under such conditions, a two-electron reversible reduction occurs, with the uptake of two atoms of hydrogen per molecule of adrenochrome. The same hydrogen uptake was obtained on reduction of the analogous pigment formed by the oxidation of epinine ($C_6H_3(OH)_2CH_2CH_2NHCH_3$), thus ruling out the possibility that reduction of the hydroxyl group in the closed side chain of adrenochrome might have been responsible for the above findings. The present results are in agreement with the structures of the adrenochrome redox system originally proposed by Green and Richter (*Biochem. J.* 31: 596, 1937).

Elimination of enzyme diffusion artefacts in histochemical localization of cholinesterases. GEORGE B. KOELLE. *Dept. of Pharmacology, Columbia Univ. College of Physicians and Surgeons, New York City.*

When a histochemical procedure for localizing cholinesterases (ChE's) (*J. Pharmacol. & Exper. Therap.* 100: 158, 1950) was applied to frozen sections 10 micra in thickness, it was found, in comparison with thicker sections, that the degree of staining at certain sites was markedly diminished, whereas the relative intensity of nuclear staining was accentuated in several tissues. In contrast with the histochemical picture, Warburg determinations of the non-specific ChE activity of isolated liver cell nuclei indicated that they contained comparatively low concentrations of the enzyme. It appeared, therefore, that substantial portions of the ChE's diffused from their original sites during the course of incubation and were partially fixed at the nuclear membranes. Studies were conducted with purified preparations of specific and non-specific ChE to determine the conditions under which complete precipitation could be produced without marked impairment of enzymatic activity. As a result, the original incubation media for the histochemical method were modified by buffering at pH 6.0 and incorporating a final concentration of 20% sodium sulphate to fix specific ChE or 24% for non-specific ChE. Sections of the cat stellate ganglion so treated showed most of the specific ChE activity to be localized in the preganglionic fibers, protoplasmic tracts and cytoplasm of isolated ganglion cells; the greatest concentrations of non-specific ChE appeared to be in the cytoplasm of the capsular glial cells. The nuclei showed little staining for either enzyme. Findings in other tissues will be presented.

Acute effects of oral gluconates in diabetes. RUDOLF KOSTER. *Dept. of Pharmacology, Tulane Univ., New Orleans, La.*

Oral sodium gluconate reduced ketones and was twice followed by recovery from diabetic coma (SCHWARZ. *Prager Med. Woch.* 26: 361, 1901). Blood sugar dropped after intestinal absorption of gluconates (JOHNSON. *Am. J. Phys.* 113: proc. 72, 1935; MITCHELL *et al. J. Nutrition* 18, 1939). Alkalinity followed oral ammonium gluconate and gluconic acid (CHENOWETH *et al. J. Lab. & Clin. Med.* 26: 1574, 1941). Weight, Purina Lab Chow consumption, water, and urine analysis were recorded for 24-hour periods on 13 young adult diabetic Carworth rats. Thirteen days after alloxan, gluconate solutions replaced water for 3–6 days, each. Concentration proved more significant than dose; effects are delayed. Urines below pH 6 reached pH 9 on the second day after doses of 8% (2 gm/kg.) or 10% (8 gm/kg.) sodium gluconate, despite subnormal intakes of fluid, whereas much larger doses at lower concentrations, respectively, were ineffective. Alkalinity persisted one day after withdrawal. Ketones (Rothera Test) disappeared after 3 gm/kg. of 8% sodium salt, 3

gm/kg. of 5% potassium salt, 1 gm/kg. of 5% acid, or 5 gm/kg. of 4% acid. Urine sugar was unchanged by up to 30 gm/kg. of 7% sodium gluconate, but disappeared on the second day after 10–30 gm/kg. of 8%, and sometimes after 3 to 10 gm/kg. of 5% potassium salt. Sugar re-appeared one day after drug withdrawal. Food, weight, and urine volume dropped. Diarrhea never developed. Rats refused calcium 2-, calcium 5-, and sodium 2-ketogluconate. Intravenous gluconic acid, 0.1 gm/kg., 10%, reduced fasting blood sugar in normal rabbits for 2 hours. (Potassium gluconate, sodium gluconate, and acid donated by Pfizer and Co.; alloxan and ketogluconates, by Hoffmann-La Roche.)

Effect of thiopental and pentobarbital on contractions of normal, denervated and curarized mammalian muscle. CHARLES P. KRAATZ, MELVYN I. GLUCKMAN* AND HUBERT L. SHIELDS.* *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

The contractions of the anterior tibial muscle in normal and denervated (6–12 days) dogs under urethane anesthesia and in decerebrate cats were recorded. The tension of the maximal twitch of normal dog muscle was increased 22% by the rapid intraarterial (femoral) injection of 1 mg/kg. of thiopental sodium, the usual experimental dose. A measurable increase was evoked by 0.125 mg/kg. Denervated muscle was potentiated to about two-thirds the extent of normal muscle, while cat muscle showed a somewhat greater potentiation of the twitch. Thiopental augmented the initial tension of tetanized muscle at frequencies below 100/second and decreased it at higher frequencies. The tension however declined more rapidly with all frequencies. Post-tetanic potentiation was further augmented by thiopental. Low levels of curarization (nerve stimulated at 6 or 30/minute) were completely antagonized by thiopental while full curarization was unmodified. The antagonism was reversed in the dog by increasing the duration of the stimulus to 5 msec. or the frequency to 2/second. The incidence of 'head-drop' in the rabbit from threshold doses (0.14 mg/kg.) of *d*-tubocurarine chloride was markedly reduced by the prior intravenous administration of 5 mg/kg. of thiopental sodium. Pentobarbital sodium did not antagonize curare on tibial twitches but equalled thiopental in antagonizing curare 'head-drop.' Otherwise, pentobarbital duplicated the actions of thiopental with about half the activity.

Quinine-like action of veratramine upon the single twitch and upon the 'veratrine response' of the sartorius muscle of the frog. OTTO KRAYER. *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

In concentrations of 1:100,000 veratramine increases the twitch height of the isolated sartorius muscle suspended in a bicarbonate buffer solution and stimulated by electric shocks of 40 volts and 0.5 millisecond duration. Concentrations of 1:30,000 and higher abolish the responsiveness to the electrical stimulus after a transient increase in twitch height. Veratramine does not cause a myotonic response in a concentration of 1:100,000; while concentrations of 1:30,000, and higher, in some experiments lead to a light retardation of relaxation after the twitch. The 'veratrine response' caused by veratridine (1:10,000,000-1:5,000,000) can be antagonized or prevented by veratramine in concentrations of 1:100,000. The effect of veratramine upon twitch height as well as its antagonistic action to the veratrine response are qualitatively and quantitatively similar to the well-known actions of quinine and quinidine.

Evaluation of the central action of anticholinesterases in producing respiratory paralysis. WILLIAM A. KRIVOY* AND AMEDEO S. MARRAZZI. *Toxicology Section, Med. Div., Army Chemical Center, Md.*

The respiratory paralysis produced by large doses of DFP and other anticholinesterases may come about through the secondary depression of either or both the neuroeffector junctions and central nervous system synapses. If an effective outflow of impulses in the respiratory nerves would outlast respiratory paralysis, then the peripheral effect would appear to be the determining factor. An analysis of action potentials recorded from the phrenic and intercostal nerves after large doses of DFP will be presented and correlated with records of respiration obtained from the trachea and diaphragm.

Effect of glyoxal on tissue metabolism. ERNEST KUN. *Dept. of Medicine, Univ. of Chicago, Chicago, Ill.*

Glyoxal, similar to methylglyoxal (*J. Biol. Chem.* 187: 289, 1950) inhibits enzymes requiring -SH groups for their catalytic activity. The oxidation of pyruvate by rat brain slices and mitochondrium preparations, brain and muscle triose phosphate dehydrogenase, and hexokinase are inhibited 40–90% when the enzyme preparations are incubated with glyoxal ($5 \times 10^{-5} - 5 \times 10^{-3}$ M final glyoxal concentration). The esterification of inorganic P during the anaerobic dismutation of Harden Young ester by brain and muscle extracts is greatly inhibited by glyoxal. Glyoxal is enzymatically converted to glycolic acid under anaerobic conditions by rat liver extract in presence of GSH. The substrate of this enzyme was isolated and identified as $C_2O_2H_2(GSH)_2$. Glycolic acid is further oxidized by an enzyme present in rat liver extracts to glyoxylic acid. This oxidation does not require DPN. The enzyme has been char-

acterized as a soluble flavoprotein. Rat brain, kidney, heart muscle and Flexner Jobling rat carcinoma does not contain this enzyme. It is present in appreciable quantities in the liver.

Quantitative studies on the potentiation of barbiturate anesthesia by glucose and by sodium lactate. PAUL D. LAMSON, MARGARET E. GREIG AND CHARLIE J. HOBDY.* *Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

The potentiation of hexobarbital anesthesia in guinea pigs with glucose and with sodium lactate, and in mice and rats with sodium lactate has been studied by determining the reduction of the minimum anesthetic dose of hexobarbital by these substances. The level of hexobarbital in the brain at which anesthesia was produced with hexobarbital alone, with hexobarbital and glucose, as well as with hexobarbital and sodium lactate was also determined by spectrophotometric means.

Complement titres in different phases of nephritis. KURT LANGE, FRANK GRAIG, JACOB OBERMAN AND FRED LOCASTO (introduced by M. G. MULINOS). *Dept. of Medicine, New York Med. College, New York City.*

Complement of serum was examined using·50% hemolysin as point of reading. Commerical guinea pig complement with inactivated serum was used as a standard. The results were calculated in terms of complement units. Normal sera and sera from various chronic and febrile diseases, including cases of rheumatic fever, were found to have a range between 0.9 and 3 complement units, with an average of 1.7. Acute and subacute nephritics all showed very low complement content ranging between 0 and 1.0 complement units with an average of 0.38. Apparently healed cases showed normal values ranging from 1.3–2.6 complement units with an average of 1.6. Cases of the nephrotic state varied in their findings, some having normal complement. A few others had values similar to those found in acute nephritis. Recovery in acute nephritis was associated with a gradual rise of complement to normal. One severe acute nephritic which ended fatally showed persistently complete absence of complement. This tends to confirm our previous studies where we showed glomerulonephritis to be due to an antigen-antibody reaction.

Cholinesterase, choline oxidase and tributyrinase activity of rat liver and rat liver tumors. H. LANGEMANN* AND C. J. KENSLER. *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

Viollier (*Helv. Physio. Acta* 8: C37–41, 1950) has reported that the cholinesterase activity was increased and that choline oxidase and tributyrinase activity was decreased in rat liver tumors produced by feeding N,N-dimethyl-p-aminoazobenzene (DMB). In the present study the activity of these enzyme systems in rat liver and rat liver tumors produced by feeding 3′methyl-N,N-dimethyl-p-aminoazobenzene (3′M-DMB) have been compared. Choline oxidase and tributyrinase activity in the tumors was uniformly 10% or less of that found in liver, whereas cholinesterase activity was in general, though not always, higher in the tumors. As in other species tributyrinase activity and 'non-specific' cholinesterase activity have been reported to parallel one another, these results suggested that 'specific' cholinesterase was selectively increased. Using the rates of hydrolysis of benzoylcholine and mecholyl to differentiate between non-specific and specific cholinesterase, it was found that either or both types were increased in individual tumors. Different tumors, even from the same animal, showed widely different activity and different distribution of activity between the two types of cholinesterase. Tributyrinase activity did not parallel non-specific cholinesterase activity. Following the separation of rat liver by centrifugation in 0.88 M sucrose into nuclear, large granule (mitochondria) and supernatant fractions, 70% or more of the cholinesterase and tributyrinase activity was recovered in the supernatant fraction and approximately 70% of the choline oxidase activity was recovered in the mitochondria fraction.

Observations on the nicotinolytic activity of some aralkyl amines. P. S. LARSON, C. B. VAN SLYKE,* J. K. FINNEGAN AND H. B. HAAG. *Dept. of Pharmacology, Med. College of Virginia, Richmond.*

Twelve compounds of the general formula (R^1, R^2, R^4) C-R^4-CH_2-CH(R^5)-N(R^6, R^7) have been tested for nicotinolytic activity as judged by their ability to antagonize the pressor effect of nicotine on dogs. In order of decreasing potency, these compounds were: 6-dimethylamino-4,4-diphenyl-3-heptanone [methadone]; N,N,1-trimethyl-3,3-diphenyl-4-hexenylamine; 2-(benzhydryloxy) triethylamine; N-methyl-N-(3,3-diphenyl-sec-butyl) butylamine; N-ethyl-1-methyl-3,3-diphenylpropylamine; N,N-dimethyl-3,3-diphenyl-sec-butylamine, 2-(benzhydrylmercapto)-N,N-dimethylethylamine, α-(benzhydrylmethyl)-N,N-dimethylbenzylamine and 2-[p-chloro-α-(2-dimethylaminoethyl)benzyl] pyridine-6-dimethylamino-4-phenyl-4-(3-pyridyl)-3-hexanone; N,N-dimethyl-3,3-diphenylpropylamine; 2-(benzhydryloxy)-N,N-dimethylamine [diphenhydramine]. The i. v. dose required to effect a 50% reduction in the pressor response to 0.02–0.03 mg/kg. nicotin given i. v. ranged from 0.2 mg/kg. for methadone to greater than 40 mg/kg.

for diphenhydramine. The pressor response to epinephrine was potentiated by some compounds and somewhat reduced by others. (This study was made under a research grant from the American Tobacco Company. The compounds, excepting methadone, were kindly supplied by Parke, Davis & Company.)

Effect of carbon tetrachloride on toxicity and storage of methoxychlor in the rat. EDWIN P. LAUG AND FRIEDA M. KUNZE.* *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Earlier experiments have shown that methoxychlor has a low order of toxicity and exhibits little tendency for accumulation in the tissues. Under the influence of carbon tetrachloride given concurrently in oral doses of 0.5–1.0 ml/kg., methoxychlor produces not only toxic symptoms similar to the characteristic tremors observed in DDT poisoning but also tends to accumulate in the abdominal fat and the liver in amounts from 10–100 times as much as in the control tissues. The rôle of liver injury and its possible influence on the metabolism of methoxychlor will be considered.

Occurrence of nor-epinephrine in toad venom. HENRY M. LEE AND K. K. CHEN. *Lilly Research Labs., Indianapolis, Ind.*

It has been firmly established that epinephrine of adrenal medulla contains various amounts of nor-epinephrine. Epinephrine also occurs in toad venom, a product of the skin, as shown by Abel and Macht with *Bufo marinus*, and by Jensen and Chen with Ch'an Su which is a commercial preparation of toad venom. In order to ascertain if epinephrine of toad venom might be also admixed with nor-epinephrine, experiments were carried out with a specimen prepared from Ch'an Su by one of us (K. K. C.). Biological tests failed to show any presence of nor-epinephrine. It was not until paper chromatography was employed that evidence for nor-epinephrine was obtained. After chromatographic separation on a broad sheet of Whatman No. 1 filter paper, nor-epinephrine could be eluted and identified biologically by the method of blood pressure and nictitating membrane in the anesthetized cat. When compared with known quantities of nor-epinephrine on the chromatogram the amount present in the sample of epinephrine of Ch'an Su was found to be approximately 2–4% calculated as the bitartrate (1–2% as the base).

Oxygen consumption of normal and denervated muscle in the presence and absence of glucose. KWANG SOO LEE,* CHARLES M. GRUBER, JR., CHARLES M. GRUBER AND ZINAIDA T. LASZICZENKO.* *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

A comparison was made of the oxygen consumption of normal and denervated muscle as measured by the Warburg technique with special reference to the effects of glucose in the system. Denervation of the rat diaphragm was accomplished by destroying the phrenic nerve. Only one side was denervated the other side remaining intact to serve as a control and animals were sacrificed 10–14 days after denervation. Using Krebs-Ringer solution without glucose, 21 experiments were done on 126 rats employing 14 vessels in each experiment. Nine experiments were done on 54 rats in which the rat diaphragms were immersed in Krebs-Ringer solution with 0.2% glucose. In the experiments without glucose the average Q_{O_2} during the first half hour was for normal diaphragm 6.48 and for denervated diaphragm 7.66, showing about 18% increased oxygen consumption for denervated muscle. In the experiments with 0.2% glucose the average Q_{O_2} during the first half hour was 8.48 for normal and 8.19 for denervated diaphragm. The difference between these is statistically significant.

Thorn test in institutionalized schizophrenic patients. ROBERT E. LEE,* CARVELLE BABCOCK,* RICHARD REILLY,* LOUIS SCHLANN* AND CARL C. PFEIFFER. *Dept. of Pharmacology, Univ. of Ill. College of Medicine, Chicago, and Manteno State Hosp.*

Two groups of 20, and 6 schizophrenic patients respectively were divided into equal subgroups on the basis of their eosinopenic response to epinephrin (0.5 mg.) and ACTH (25 mg.). Fifty % of each group were chosen because they did not respond to the Thorn test (non-responders). The remaining half responded in a normal manner with at least a 40% drop in the eosinophil count at 4 hours after 0.5 mg. of epinephrin (responders). However, over an experimental period of 10 weeks neither category of response remained absolute when Thorn tests were repeated two to three times per week. The number of non-responding patients increased under stress therapy. Niacinamide and thyroid produced the opposite effect in that many of the non-responders became responders on these medications.

Experimental medionecrosis of the arterial tree and its prevention by ammonium chloride. DAVID LEHR. *Dept. of Pharmacology, The New York Med. College, Flower and Fifth Avenue Hosps., New York City.*

Medionecrosis and calcification of the aorta and larger branches was produced in albino rats by the administration of sparingly soluble sulfonamides in excessive dosage. The arterial lesion appeared invariably linked to obstructive nephropathy resulting from massive intratubular crystalline deposition of these sulfonamides or their

metabolites. The significance of the renal injury in the development of arterial lesions was investigated, therefore, in experiments designed either to inhibit renal blockage by alkalization or to enhance it by acidification. It was found that adequate protection of the kidney with sodium bicarbonate, prevented the development of arterial damage. If sodium chloride, which had an acidifying effect, was used instead of sodium bicarbonate, substantial aggravation of the renal injury was observed, if compared with the kidneys of untreated sulfonamide control animals. In addition, the sodium chloride treated rats exhibited most consistently the severest degrees of aortic mediosclerosis with true bony metaplasia in some instances. Substitution of sodium chloride by the stronger acidifier, ammonium chloride, on the other hand, resulted in partial or complete prevention of the vascular lesion although obstructive nephropathy was obviously not averted by the use of ammonium chloride. The protective action of this salt against arterial injury reported for the first time eight years ago (D. LEHR. *Bull. New York Med. Coll.* 6: 70, 1943) and substantiated in many subsequent studies, has since been confirmed by other authors in similar though differently induced arterial lesions (Selye, H. *et al. Lancet* 1: 301, 1945; MCLEAN, C. R. AND R. H. MORE. *Proc. Soc. Exper. Biol. & Med.* 71: 648, 1949). The contributory influence of hemodynamic, dietary, hormonal and mineral metabolic factors in the development of arterial mediosclerosis is at present under investigation. (Supported in part by a research grant from the Natl. Heart Inst., of the Natl. Insts. of Health, Public Health Service.)

A constant flow perfusion system. J. T. LITCHFIELD, JR., MAXINE ADAMS,* MARIAN JAEGER,* AND CATHERINE TOY.* *Stamford Research Labs., American Cyanamid Company, Stamford, Conn.*

Studies on isolated guinea pig lungs perfused by the method of Sollmann and Von Oettingen (1927), later modified by Tainter *et al.* (1934), indicated the desirability of developing a new method for studying reactions of bronchi. The above-mentioned method operates on the principle that, if fluid is perfused at a constant pressure, the flow rate will vary inversely as the resistance of the bronchi, the latter being determined by the degree of constriction of the bronchial system. Thornton (1932) investigated a system in which both flow rate and perfusion pressure varied according to changes in bronchial resistance, but aside from more convenient recording, this system offered few advantages. We explored a system operating on the principle that, if flow rate is constant, the perfusion pressure will vary directly with changes in bronchial resistance. A constant flow non-pulsating pump driven by air pressure was developed. Perfusion pressures were recorded by means of a small tambour and an optical recording system. Guinea pig lungs were prepared for perfusion by conventional methods. The perfusion fluid was balanced salt solution, not containing histamine or any similar stimulant. Responses to physiologic quantities of drugs were readily obtained, e.g. effective doses in micrograms were: epinephrine 0.1, butanephrine 1.0, theophylline 200, acetylcholine 0.05, histamine 1.0. Thus, the sensitivity is 100–1000 times greater than previous systems using perfused lung. The usable life of such a lung preparation is in excess of 3 hours. The perfusion system has been found useful also for studying vascular reactivity of isolated organs. The constant flow pump has also been used for the administration of intravenous fluids.

Prevention of drug-produced ejaculation by autonomic ganglionic and adrenergic blockade. S. LOEWE AND SUSANNE L. PUTTUCK.* *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

Drug-elicited ejaculation in the mouse (LOEWE. *J. Pharmacol.* 63: 70, 1938), which under optimal conditions is invariably successful, has proved to be useful in the study of the nervous pathways of the ejaculatory process (LOEWE. *Bull. XVI. Internat. Physiol.* Congress, Zürich, p. 145, 1938). Studies on the rôle of autonomic components demonstrated a limited facilitation by cholinergic drugs and some inhibition by large doses of atropine; adrenergic drugs neither facilitated nor inhibited the ejaculatory response. The participation of sympathetic functions has now been studied by the aid of ganglionic and adrenergic blocking agents. After adequate doses of appropriate adrenolytics, the ejaculatory response was completely suppressed, e.g. by 2 mg/kg. of "SKF 688 A" (N-benzyl-N-phenoxyisopropyl-beta-chloroethylamine) injected intramuscularly 20 hours or by 20 mg/kg. Regitine (N-*p*-tolyl-N-*m*-hydroxyphenylaminomethylimidazoline) 15 seconds prior to the drugs used to elicit ejaculation. The inhibitory activities of a variety of sympatholytics were proportional to their known adrenergic blocking potencies. Autonomic ganglionic blocking agents were also effective anti-ejaculants. C6 (1,6-bis-ammonium hexamethylene), which selectively blocks sympathetic ganglionic transmission, was approximately equieffective with the non-selectively blocking agent tetraethylammonium. All findings are compatible with the concept that the ejaculatory efferent system is sympathetic and that the impulses to the effector organs are adrenergic.

Distribution of 2,4-diaminopyrimidine antimalarial in the tissues of the dog. DORIS C. LORZ, PHOEBE LEE GRAHAM AND GEORGE H. HITCHINGS (introduced by E. J. DE BEER). *Wellcome Research Labs., Tuckahoe, N. Y.*

The blood and tissue levels of several 2,4-diaminopyrimidine antimalarials could be determined by means of the dye-lake method of Marshall, with minor modifications. After repeated daily or twice daily doses, either oral or intravenous, the blood levels rose steadily during the first week then approached equilibrium. Only a small proportion of the substance was excreted unchanged. On sacrifice of the animals, the tissues were found to contain rather high levels of the pyrimidine. In liver, spleen, brain and bone marrow, especially, concentrations several-fold higher than those of the blood were found, while the content of kidney and muscle was low. When administration of the compound was discontinued the blood levels fell slowly, the die-away curve indicating a half-life of about 1½ days.

Concerning the effects of sympathomimetic agents on coronary flow and heart action, recorded simultaneously. F. C. LU* AND K. I. MELVILLE. *Dept. of Pharmacology, McGill Univ., Montreal, Quebec, Canada.*

The inter-related changes in coronary flow rate and heart contractions in isolated rabbit hearts were studied by a method recently described (*J. Pharmacol.* 99: 277, 1950). The data obtained show, viz.: 1) *Ephedrine* (0.01–0.1 mg.) exerts no effect on coronary flow nor heart action, but larger doses (1–10 mg.) induce initial myocardial depression associated with an increase flow, progressive cardiac stimulation then follows during which the flow is mechanically decreased, but subsequently secondarily increased. 2) *Phenylephrine* (Neosynephrine)—0,0003–0.008 mg.—induces transient coronary vasoconstriction, but no change in heart contractions. Larger doses (0.04–5 mg.) produce myocardial stimulation associated with an initial greater decrease in flow followed by a secondary increase. 3) *Isopropylaterenol* (Isuprel) in small doses (0.0000024–0.000012 mg.) leads to both myocardial stimulation and increased coronary flow. Larger doses (0.00006–0.04 mg.) induce marked stimulation usually associated with an initial decrease followed by prolonged secondary increase. 4) *Methoxamine* (Vasoxyl)—0.01–0.1 mg.—exerts no effect on coronary circulation and no change in heart action (rate or amplitude). Larger doses (1–10 mg.) induce transient myocardial depression associated with transient increase in flow. This was the only sympathomimetic agent tested which did not induce associated myocardial stimulation. These findings confirm in general our earlier studies on epinephrine and nor-epinephrine, and indicate clearly that changes in coronary flow (increase or decrease) induced by sympathomimetic agents are essentially *secondary* to the associated changes in heart action.

Diethylether and chloroform on brain ATPase. GO LU, J. S. L. LING,* C. JELLEFF CARR AND JOHN C. KRANTZ, JR. *Dept. of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.*

By using a modified DuBois and Potter method, the ATPase activity in rat brain was compared under normal conditions and with certain volatile anesthetics. Preliminary screening tests showed that diethylether (250 mg.% or greater) and chloroform (50 mg.% or greater) depressed the ATPase activity to a significant degree. Lower concentrations gave variable results. It is possible that such a diminution of ATPase activity by anesthetics will result in decrease of functional activity of brain cells. Consequently, this fact would be of importance in explaining the mechanism of general anesthesia.

Therapeutic efficacy of 'blood substitutes' in experimental traumatic shock. LEHMAN M. LUSKY* AND HERBERT A. BRAUN. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Rabbits were anesthetized with pentobarbital sodium and tourniquets were applied to the hind legs of the animals for 5 hours. Comparable groups of these shocked animals were treated intravenously, 8 cc/kg., with solutions of Dextran, 6%; Periston, 3.5%; and dextrose, 10% in normal saline. Treatment was initiated one minute after release of the tourniquet and repeated in 24 hours. The animals were kept under observation for 120 hours and all deaths within this period were recorded. The number of animals surviving this period was selected as the most useful measure of the therapeutic efficacy of the blood substitute administered. We were unable to demonstrate a significant difference in the therapeutic efficacy of Dextran, Periston or dextrose in saline using the above technique of inducing shock with this dosage and schedule of treatment.

Evaluation of drugs for possible use in therapy of auricular fibrillation. E. L. MCCAWLEY, G. A. WESTON* AND H. L. H. DICK.* *Dept. of Pharmacology, Univ. of Oregon Med. School, Portland.*

Because there is no complete agreement of the nature of the mechanism underlying auricular fibrillation, a variety of pharmacologic procedures are needed in evaluating therapeutic agents for use in this disorder. The isolated rabbit auricle has been adapted by Dawes to screen drugs which can prolong the 'effective' refractory period of atrial myocardium. We have been able to induce

fibrillation in the isolated auricle by production of an artificial infarct or injection of aconitine. This technique is also useful for screening purposes. The importance of vagal activity in establishing and maintaining clinical fibrillation has been stressed repeatedly. In the dog, antivagal properties may be determined in experiments involving administration of methacholine or stimulation of the vagus. The vagal chronotropic cardiac action can be altered by antifibrillatory drugs without reversing the peripheral vascular effects. Auricular fibrillation can be mimicked in dogs by producing ectopic foci from anoxic infarcts, aconitine or with high frequency electrical impulses. Vagal stimulation (digitalis) may be added to these techniques to establish persistent fibrillation. Quinidine and certain antihistminics have been studied using these various techniques. As a result of these studies, diphenhydramine has been proven of therapeutic value in preliminary trial in patients with auricular fibrillation.

Detoxification of phytotoxic sera by cortisone and ACTH. DAVID I. MACHT. *Dept. of Pharmacology, Labs. of Sinai Hosp., Baltimore, Md.*

The author described in numerous previous papers the phytotoxic action on root growth of Lupinus Albus Seedlings under standardized ecological conditions by sera of the following diseases: menstruation, pernicious anemia, pemphigus, and various psychoses (*Bull. Torrey Botan. Club* 76: 235, 1949). Experiments were made with aqueous extracts of suprarenal cortex (Upjohn) in the summer of 1948. It was found *in vitro* that these destroyed the toxin of pemphigus when studied by above methods. Experiments were recently undertaken with cortisone solutions *in vitro* on the phytotoxic reactions of the above sera. As little as 1 to 5,000,000 of cortisone produced marked detoxification of pemphigus and psychotic sera, but had no effect on toxicity of menstrual and P. A. sera. Elsewhere the author described a specific phytotoxic effect of psoriasis serum on 'vernalized' or 'yarovized' seedlings. (*J. Invest. Dermat.* 13: 1, 1949). This was found also to be detoxified by cortisone. ACTH produced exactly the same effects, in all of above conditions. In case of pemphigus the findings above ran exactly parallel to phytopharmacological examinations of blood from patients before and after treatment with cortisone or ACTH.

Estimation and metabolism of lidocaine. F. G. MCMAHON* AND L. A. WOODS. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Lidocaine (Xylocaine, α-diethylamino-2,6-acetoxylidide) has recently been introduced as a local anesthetic of wide application. The chemical structure of this drug suggests that its metabolism by living tissues might be expected to be slow, if it occurs at all. Using a modification of the method of Woods *et al.* (*J. Pharmacol. & Exper. Therap.* 101, in press), the estimation of lidocaine in plasma, urine and tissues was investigated. Average percentage recoveries for ten determinations at each level of lidocaine added to oxalated dog plasma in concentrations of 1–25 μg/ml. varied from 106 [C (coefficient of variation = $s/\bar{x}$) of 14%] at the lowest level to about 99.0 [C of about 2.5%] at the higher concentrations. Similar results were obtained on dog urine. Percentage recoveries of 50 μg/gm. of lidocaine from various tissues of the albino rat ranged from 90–109%. Female mongrel dogs were given 15 mg/kg. of lidocaine (free amine). Following intravenous administration plasma levels gradually decline over a period of 6 hours from the peak plasma level (initial sample, 3–6 minutes after injection) of about 5.5 μg/ml.; about 10% of the dose appears in the 24-hour urine. After subcutaneous administration plasma levels reach a maximum of about 5.1 μg/ml. at 90 minutes following injection and decline to zero in about 6 hours; approximately 20% of the drug is recovered in the 24-hour urine. Analysis of tissues indicates that no selective tissue deposition occurs following the intraperitoneal injection of 100 mg/kg. of lidocaine into albino rats.

Neuromuscular effects of DFP. B. P. MCNAMARA, A. D. BERGNER,* E. M. ROBINSON,* E. F. MURTHA* AND J. H. WILLS. *Med. Div., Army Chemical Center, Md.*

DFP (10–50 mg/kg., I.M., in oil) produces a reversible, inhibitory effect on the amplitude of the nerve action potential and muscular response (*in vivo*) of nerve-muscle preparations in cats. It also produces an irreversible effect on cholinesterase (as determined by the Koelle histological method) and on the ability of the muscle to maintain tetanus when indirectly stimulated. Evidence is presented which indicates that part of the neuromuscular effects is due to actions other than that on the esterase.

Ergotamine-like actions of ergonovine. F. J. MACRI (introduced by T. KOPPANYI). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

In a previous communication it was shown that ergonovine maleate potentiated the pressor effects of epinephrine and nor-epinephrine and reversed the depressor effects of isuprel. In order to test the specificity of ergonovine on the hemodynamic effect of isuprel, the action of the former on the vasodepressor effects of ACh and sodium nitroprusside was investigated. It was found that when ergonovine was given in doses up to 0.8 mg/kg. it reversed the depressor effect of isuprel, but had

no effect on the vasodepression produced by ACh and sodium nitroprusside. It may be concluded, therefore, that ergonovine has a specific effect on isuprel vasodepression and does not diminish cholinergic or smooth muscle vasodepression. The duration of the ergotamine-like action of ergonovine was also determined at two dosage levels (0.1 mg/kg. and 0.6 mg/kg.). The lower dose lasted for a period of about 3½ hours. At the end of this period both the epinephrine—and the isuprel-vasodepressor responses returned to pre-ergonovine levels. When, however, the higher dose was employed, the isuprel responses returned to normal in approximately 5 hours, but the epinephrine potentiation outlasted this isuprel reversal by several hours. Therefore, at this time, it cannot be stated with certainty whether or not the ergonovine effects on isuprel and on epinephrine are due to exactly the same mechanism. Dogs under barbiturate anesthesia were used throughout these experiments.

Triiodoethylate of tri (diethyl-amino-ethoxy) 1, 2, 3 benzene (Flaxedil) in anesthetized man. B. E. Marbury and Joseph F. Artusio, Jr. (introduced by Walter F. Riker). *Depts. of Surgery (Anesthesia) and Pharmacology, Cornell Univ. Med. College and New York Hosp., New York City.*

A study of the curare-like properties of the triiodoethylate of tri(diethylaminoethoxy) 1, 2, 3 benzene (Flaxedil) was carried out in 42 anesthetized patients. The method of measuring respiratory minute volume (RMV) was used as a measure of muscular paralysis (*J. Pharmacol. & Exper. Therap.* 100: 227, 237, 1950). The dose response was determined for single, intravenous doses of Flaxedil. Doses of 0.5, 1.0, 1.5, and 2.0 mg/kg. reduced the RMV to 90%, 70%, 30%, and 10% of control respectively. Maximal depression of the RMV occurred within 5 minutes. The average duration of curariform action after doses of 1–2 mg/kg. was 40 minutes. Cumulation of Flaxedil was noted when an identical dose was given immediately after the RMV returned to its control level. 3-Hydroxy phenyltrimethylammonium bromide (Ro 2-2561) was an effective antagonist to the curare-like action of Flaxedil. The antagonism of Ro 2-2561 to Flaxedil was identical to its anticurare action. The only side-effect found as a result of the use of Flaxedil was a sinus tachycardia. The duration of this effect persisted longer than that of the neuromuscular blockade.

Comparison of the physiological disposition and metabolic transformation of Nembutal and Pentothal. Lester C. Mark, Eleonore Bernstein,* J. J. Burns,* Philip A. Lief* and Bernard B. Brodie. *Depts. of Anesthesiology, New York Univ.-Bellevue Med. Center and Montefiore Hosp., New York Univ. Research Service, Goldwater Memorial Hosp., New York City, and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Nembutal is almost completely metabolized in man. A major route of metabolism involves oxidation of the methylbutyl side chain to a secondary alcohol. This finding corroborates the work of Maynert and van Dyke (*Science* 110: 661, 1949) in dogs. Nembutal thus follows a different oxidative pathway than that reported by us for the thio analogue, Pentothal, which undergoes oxidation to a carboxylic acid. We have reported previously that the anesthetic activity of a single dose of Pentothal is limited in duration by its rapid localization in fat. Experiments with dogs indicate that Nembutal, a longer-lasting anesthetic than Pentothal, is not appreciably localized in body fat. This explains, at least in part, its longer duration of action. Both drugs are distributed similarly in the various organ tissues. On the other hand, the binding of Nembutal on plasma proteins is considerably less than that of Pentothal. Interestingly enough, it is metabolized more rapidly than Pentothal in the dog. Whether this is also true for man is being investigated. The implications of the above findings with reference to the clinical behavior of the drugs will be discussed.

Cerebral synaptic action of amphetamine and ephedrine compared with that of caffeine. Amedeo S. Marrazzi and E. Ross Hart. *Toxicology Section, Med. Div., Army Chemical Center, Md.*

Though amphetamine and ephedrine are generally regarded as producing cerebral excitation, previous studies (Marrazzi, A. S. *Federation Proc.* 1: 57, 1942) of the action potentials evoked in the optic cortex of the cat suggest that these drugs are capable of interfering with the arrival at the cortex of impulses initiated in the optic tract. The problem has been restudied in the simplified situation where an impulse initiated in one optic cortex needs to traverse but one group of synapses to reach the corresponding area in the contralateral optic cortex by fibers passing through the corpus callosum. The evoked electrical activity in the contralateral cortex records simultaneously the impulses entering and leaving the cortical synapses (Marrazzi, A. S. and E. R. Hart *J. Pharmacol. & Exper. Therap.* 98: 22, 1950). This has made possible a complete analysis of the previously described cortical actions of amphetamine and ephedrine. These will be compared to the actions of caffeine and analyzed in terms of a possible release phenomenon.

Curare compounds. X. The distribution and fate of radioactive *d*-Chondocurarine and *d*-tubocurarine dimethylether iodide. David F. Marsh.

Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgantown.

Chondocurine was reacted with two equivalents of $C^{14}H_3I$ to yield *d*-Chondocurarine iodide. Part of this material was further reacted in the presence of methanolic KOH to yield *d*-O-methyltubocurarine iodide containing four radioactive carbon atoms. The labelled materials produced 160 and 314 counts per minute per microgram. The melting point, solubility, and rabbit head drop doses are the same as the unlabelled materials. They can be extracted from biological materials with ethylene-dichloride in the presence of a pH 10 buffer or paper chromatographed with water saturated amyl alcohol. Location of the material on the paper chromatographs can be accomplished by radioautography or it can be found with an ultraviolet light by means of the characteristic 280 mμ absorption band in amounts of as little as 0.1 microgram per square centimeter. Following intravenous injection of doses of either of these agents that produce minimal skeletal muscle paralysis in dogs, 90% disappears from the blood within 120 seconds. Some unchanged drug appears in the urine almost immediately with 3% of the administered dose excreted unchanged within 3 minutes and 10% in 15 minutes. Higher doses or repeated administration increases the blood level and the urinary excretory rate. No material is found in the feces. Radioactive material is present in muscle and excreted long after paralysis disappears. The effect of the drug is apparently related to the establishment of a concentration gradient at the site of action with subsequent redistribution in the body accounting for the transitory duration of action and increased response to repeated administration. The biologic half time for a single head drop dose is approximately 7 minutes with either agent.

Maintenance dose of a rapidly eliminated digitalis glycoside, digoxin. RAYMOND MARSH,* LAWRENCE HANLON,* HARRY GOLD, HAROLD OTTO,* THEODORE GREINER* AND SYDNEY MATHES.* *Dept. Pharmacology Cornell Univ. Med. College, Cardiovascular Research Unit Beth Israel Hosp., and Cardiology Service Hosp. for Joint Diseases, New York City.*

Individual patients differ in the amount of digitalis they require, but a dose can be established which is an optimum starting point for a regimen of maintenance. This is the dose which serves the highest number, and yields a reasonably low incidence of toxicity. In a group of 30 ambulant patients with auricular fibrillation and rapid ventricular rate when without digitalis, in attendance at our cardiac clinics, a daily dose of 0.25 mg. of digoxin maintained the apex rate below 85 a minute in 20% of the group, 0.5 mg. in 50%, and 0.75 mg. in 75%. In the case of the last dose, the incidence of gastrointestinal toxicity was 4%. A daily dose of 1 mg. raised the incidence of satisfactory slowing of the heart, but also increased the incidence of toxicity to 35%. In 20 of these patients, 0.75 mg. digoxin proved comparable to 0.2 mg. digitoxin for daily maintenance. These results show that for the long-acting digitoxin the daily maintenance dose is approximately 15% of the digitalizing dose, while for the short-acting digoxin, the maintenance dose is approximately 65% of the digitalizing dose.

Comparison of the sensitivity of children and adults to digitoxin. SYDNEY MATHES,* THEODORE GREINER,* CHARLES MESSELOFF,* HARRY GOLD, MORRIS PEARLMUTTER* AND RAYMOND MARSH.* *Dept. Pharmacology Cornell Univ. Med. College, Cardiovascular Research Unit Beth Israel Hosp., and Cardiology Service Hosp. for Joint Diseases, New York City.*

This report deals with an experiment to determine the relative sensitivity of children and adults to digitoxin. There is the general belief that children require more than adults in terms of body weight. This view receives its chief support from the studies of McColloch about 30 years ago, from which he concluded that children need from 50–500% more than adults. The fact that the criteria for digitalization in adults were different from those in children leaves open to question the validity of this conclusion. In the present study comparable myocardial effects in adults and children served as a basis for comparison, namely, the change in the T-wave of the electrocardiogram. Ambulant adults and children attending our cardiac clinics received a series of doses of digitoxin (a month or more elapsing between doses to insure recovery from the previous dose). The percentage of patients who showed a change in the T-wave increased as the dose in terms of body weight was increased. This procedure provided data for two dosage-response curves in which the log of the dose was plotted against the percentage of cases showing a response, one curve for adults and the other for children. They showed that children are more tolerant than adults when digitoxin is administered orally on a body weight basis, but that the difference is small and of questionable practical significance.

Comparative toxicity of a series of derivatives of phosphorus containing acids. E. L. MAYO,* C. H. HINE AND T. N. BURBRIDGE.* *Div. of Pharmacology and Exptl. Therapeutics, Univ. of California School of Medicine, San Francisco.*

The toxicity of a series of organic derivatives of phosphorus containing acids was determined following I.G. administration to mice and rats, and percutaneous administration to rabbits. The com-

pounds were classified as follows into 3 groups according to the predominant signs elicited in mice: 1) compounds with anticholinesterase activity; 2) compounds with tri-o-cresyl phosphate like activity; and 3) compounds with no observable effect other than motor depression. The most toxic compounds in the series belonged to *group* 1, though the activity of the compounds in the group varied considerably. The compounds studied are tabulated below according to general chemical type and predominant action. The table is arranged in order of decreasing toxicity for P and P derivatives.

O-P			Ch.E. INHIBITION	TRI-O-CRESYL LIKE	MOTOR DEPRESSION
—OR[1]	—OR	—OR	2		
—R	—OR	—OR	4	2	
—N<	—OR	—OR	1		
—R	—R	—OR		3	
—N<	—OR	—R		1	
—N<	—R	—R	1		
—O—	—OR	—R			1
		P			
—OR	—OR	—OR			1
—N<	—N<	—R	1		
—N<	—OR	—OR	1		

[1] R: alkyl, aryl or cyclic ring compound.

Cardiovascular effects of triethanolamine trinitrate—a preliminary study. K. I. MELVILLE AND F. C. LU.* *Dept. of Pharmacology, McGill Univ., Montreal, Quebec, Canada.*

In the course of other studies on coronary drugs, triethanolamine trinitrate was investigated. A 10% alcoholic solution was used and the effects compared with a similar solution of nitroglycerine. 1) On the isolated perfused rabbit heart using the procedure recently described (*J. Pharmacol.* 99: 277, 1950), it was observed that with small doses (0.008–0.2 mg.) of each agent, triethanolamine induces generally more intense and prolonged coronary dilatation with less myocardial depression (amplitude and rate). With higher doses (1 mg. of each) nitroglycerine induces greater initial dilatation but also greater initial depression, but the duration of the increase in flow was generally more prolonged with triethanolamine trinitrate. With 5 mg. of nitroglycerine cardiac arrest without recovery generally ensues, while the same dose of triethanolamine trinitrate produces temporary depression with recovery. Repeated injections of either agent usually induce some degree of tolerance. 2) On blood pressure of etherized or pentobarbitalized rabbits, cats and dogs, triethanolamine trinitrate injected intravenously exerts ten to twenty times less depressor response than nitroglycerine, dose for dose. 3) On the electrocardiogram (Lead 11) both agents produce rather similar effects, except that T wave elevation and cardiac acceleration are generally less marked with triethanolamine trinitrate, dose for dose. 4) In the dog heart-lung preparation, 5 mg. of each agent produces a brief initial increase followed by a decrease in cardiac output. These preliminary results suggest that the actions of the two agents are rather similar except that the more soluble triethanolamine compound exerts a somewhat more prolonged coronary vasodilatation, but apparently less marked myocardial and peripheral depressant actions.

Treatment of chronic leukemia with tri-ethylene melamine. LEO M. MEYER, ARTHUR SAWITSKY, MILTON R. BEYERS, NORTON D. RITZ AND CONRAD BRAHIN (introduced by ARTHUR C. DE GRAFF). *Goldwater Memorial Hosp. and Bronx V. A. Hosp., New York City.*

Seventeen adults with chronic leukemia were treated with 10–125 mg. of tri-ethylene melamine for periods of 10–200 days. Individual courses varied from 10–20 mg. Nausea, anorexia or vomiting was observed in 10 patients. Diarrhea was reported once. Hemoglobin and erythrocytes rose in 2 persons. Platelets were unaltered. No bleeding tendency occurred. Transient leukopenia was noted three times. There was eosinophilia of 3–22% in 8 cases. Leukocytes fell in 13 individuals. Decreased cellularity of bone marrow followed in 11 patients. Smears of peripheral blood and bone marrow were typically leukemic in these patients. Complete hematologic and bone marrow remission occurred in 2 cases with chronic lymphocytic leukemia. Pancytopenia with aplasia of bone marrow resulted in death following 1 course of 20 mg. In 11 instances of lymphadenopathy 5 showed decrease in size of nodes while 6 remained unchanged. Among 9 cases of hepatomegaly 4 cases had regression of liver, 1 became larger, and 4 were unchanged. Of 16 instances of splenomegaly 6 remained unaltered while 10 decreased in size. Clinical remission was excellent in 4 cases, moderately good in 6, and unimproved in 6.

Action of TEAC on the dog ileum in the presence and absence of distension. FREDERICK H. MEYERS (introduced by ROBERT A. WOODBURY). *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

Motility of the terminal ileum of the dog was

recorded by two methods. Biebl (sub-cutaneous) Loops studied with and without anesthesia but with motility recorded from a balloon on the surface provide a record of intestinal motility without distension. Water-filled balloons were placed in the lumen of the terminal ileum under pentobarbital anesthesia and distended by a pressure of 15–22 cm. of water. The activity of Biebl Loops is inhibited by tetraethyl ammonium chloride (TEAC) but the intestine distended by a water-filled balloon responds with an increase in frequency and strength of contraction as well as an increase in resting tone. The effect of TEAC in augmenting the motility of the distended intestine is not prevented by previous administration of adrenergic blocking agents, by vagal section or by atropine. Splanchnic section, however, abolishes the response. Epinephrine transiently counteracts the stimulation which follows the administration of TEAC. As an explanation for the differential effect of TEAC, it is suggested that the stimulus to activity of distension is intrinsic and not vagally mediated and that distension gives rise to afferent impulses traveling the splanchnic nerves and exciting centrally a reflex inhibition which is abolished by the sympathetic ganglionic blocking effect of TEAC.

Action of neriifolin in man. K. MEZEY AND J. PIÑEROS.* *Dept. of Pharmacology, Javeriana Univ. Med. School, Bogotá-Colombia.*

Pharmacological studies (*Federation Proc.* 8: 320, 1949) showed that neriifolin, a glycoside of Thevetia neriifolia has digitalis-like actions. Normal humans (10) tolerated well neriifolin orally in doses as 0.5–1 mg. (cat-unit: 0.2 mg.); the daily oral dose of 2 mg. produced nausea, vomiting and diarrhea. Intravenously (aqueous solution 1:2000 with 10% alcohol) 1 mg. was well tolerated; there were no signs of circulatory alterations. In 14 patients with high degree of congestive heart failure neriifolin treatment was started after a preliminary control period with salt free diet and a total fluid intake of 500–800 cc. Ventricular rate was counted three to five times daily; electrocardiographic tracings were recorded before and during the treatment; 24 hours diuresis and body weight were registered. Neriifolin was administered intravenously (7 cases) in daily doses of 0.5–2 mg. and orally, 2–4 mg. (7 cases). In all treated cases there was observed: relief from the symptoms of the heart failure, diminution of the heart and respiratory rate, increase of the diuresis, diminution of the body weight. The cardiotonic effects start to 8 hours from the beginning of the administration. Electrocardiographically signs of 'digitalization' were observed: prolongation of the P-R, depression of ST in an early stage (12–24 hours) of the treatment. These results were obtained with total doses of 5–8 mg. intravenously or 10–14 mg. orally. As to the persistence of action, symptoms of failure reappeared 5–6 days after finishing the treatment.

Effects of dextran and some other plasma substitutes in traumatic shock in mice. R. CARL MILLICAN,* SANFORD M. ROSENTHAL, ROBERT W. MOWRY* AND E. F. STOHLMAN. *Natl. Insts. of Health, Bethesda, Md.*

The acute toxicities of preparations of dextran (6%), modified gelatin (5% oxypolygelatin), polyvinylpyrrolidone (3.5%), and human serum albumin (5%) were determined in normal mice by the intravenous administration of 1 ml. doses at hourly intervals. A fatal degree of traumatic shock was produced by burns and tourniquets with standardized procedures previously described (*Pub. Health Rep.* 57: 1923, 1942; 58: 1429, 1943). Therapy was administered within an hour after the trauma, comprising 5% of body weight intravenously. The compounds were administered in 0.9% saline or 5% glucose, and compared on a mortality basis with mice receiving saline or glucose alone. In shocked mice on a mortality basis no significant therapeutic effect from these preparations could be demonstrated when compared to glucose or saline treated controls employed alternately in the same experiments. The tolerance of some of these preparations in shocked mice was considerably reduced particularly in burned animals. This was manifest by an increased mortality above that of control groups treated with saline. Normal mice manifested a toxicity to oxypolygelatin, polyvinylpyrrolidone, and a dextran preparation when administered volumes equivalent to 5–15% body weight. No toxicity was observed in normal mice administered similar volumes of another dextran preparation. Pathological and biochemical studies are being made to establish the basis for this toxicity.

Evaluation of histamine liberator activity, illustrated by a thiophanium compound RO 2-2222. RUTH MITCHELL,* PEGGY JO NEWMAN,* DOUGALD MACGILLIVRAY* AND BYRON B. CLARK. *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

In the past decade high molecular weight drugs have been shown to release histamine, but only recently has this been demonstrated with relatively simple organic compounds. This paper illustrates the methods used in testing RO 2-2222, *d*-3, 4(1′,3′-dibenzyl-2′-keto-imidazolido)-1,2-trimethylene thiophanium *d*-camphor sulfonate, a new ganglionic blocking agent, for its histamine liberator activity. When RO 2-2222 was given intravenously to dogs during preliminary screening, a vasodepressor curve suggestive of histamine release was noted. RO 2-2222, in intradermal tests,

caused typical histamine wheal and flare in humans, guinea pigs and dogs. In the only dog not showing a positive wheal, there was no histamine release on intravenous injection. This procedure apparently gives a fairly good indication of any species specificity and susceptibility. Pentobarbital anesthetized dogs were given RO 2-2222 intravenously and blood samples collected repeatedly. Plasma histamine was determined chemically and by bio-assay. The bio-assays used were response of cat blood pressure before and after atropine and before and after pyribenzamine, and the response of guinea pig ileum, these responses being compared with histamine. The micro-chemical method used, capable of demonstrating 0.1 μg/cc. was a modification of existing methods. Using another method RO 2-2222 was injected into the femoral artery and, after a latent period, systemic effects of histamine were noted. Effects were noted with doses too small to elicit any response on intravenous administration. In the latter method the animal survives while in the intravenous injection technic, the total amount of histamine liberated is so great the animal usually dies.

Psychic stress and voluntary choice of ethanol in rats. WILLIAM T. MOORE,* BERNICE M. DOUCET, HERSHEL G. TREE* AND PAUL L. EWING. *Univ. of Texas Med. Branch, Galveston.*

Performance in learning a multiple-T maze and reactions to threshold electro- and audiogenic shock are correlated with small increases in voluntary intake of 5% V/V of ethanol, under conditions in which rats are permitted free choice of water or alcohol. This behavior is interpreted as a result of psychic stress rather than a metabolic disturbance.

Cardiac protective action of Regitine (C-7337). LUCIEN E. MORRIS, CHUNG SANG YEIN, BRUNO HAID AND JOSEPH M. WHITE, JR. (introduced by ROBERT M. FEATHERSTONE). *Div. of Anesthesiology, State Univ. of Iowa Hosps., Iowa City, Iowa.*

Studies have been made of the effectiveness of Regitine (C-7337), in preventing cyclopropane-epinephrine ventricular tachycardia in dogs. Electrocardiograms were made during and following injections of the drugs and the deflections of the writing stylus were continually observed. During cyclopropane anesthesia Regitine 4.0 mg/kg. body weight consistently protected for 2 or more hours against the production of ventricular tachycardia by injection of the standard dose of epinephrine (10 gamma/kg. body weight). Preliminary clinical studies indicate that following administration of 25 mg. Regitine (total) some degree of protection may be expected against the occurrence of spontaneous ventricular irregularities during cyclopropane anesthesia. Its use in this way may be of limited value, however, because of occasional severe falls in blood pressure.

Significance of the GFR/Tm_G ratio. JOHN H. MOYER AND CARROLL A. HANDLEY. *Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

It is a generally accepted fact that glucose is transported across the renal tubular membrane in only one direction, i.e. from the glomerular filtrate into the peritubular capillaries. If tubular enzymatic activity is not altered and if the tubules of all active nephrons are transporting glucose at maximum capacity (Tm_G), then the only way in which the total amount of glucose transported by the tubules could be changed, would be to increase or decrease the number of active nephrons. Despite changes in glomerular qltration, the GFR/Tm_G should remain constant since the technique of determining Tm_G necessitates delivering more glucose to the individual tubules than they are able to reabsorb. In order to evaluate these deductions, attempts were made to alter renal function by both increasing and decreasing renal blood flow. It was increased by thyroxin and hydrazinophthalozine and decreased by hemorrhage, morphine, and epinephrine administration. Observations were made on renal blood flow, Tm_G, and glomerular filtration rate. It was observed that glomerular filtration rate could be increased or decreased significantly and was not necessarily related to renal blood flow. However, the Tm_G always paralleled changes in glomerular filtration rate. It is concluded that this was brought about by changing the number of active nephrons.

Histidinuria and liver function. JOE B. NASH,* HARVEY HAYS, JR.,* JOYCE K. HOWARD* AND G. A. EMERSON. *Univ. of Texas Med. Branch, Galveston.*

Urinary excretion of imidazoles increases in the 2d trimester of pregnancy; this has been attributed to impairment of liver function. Excretion of exogenous histidine and simultaneous blood levels were studied in dogs treated with CCl_4, and results are interpreted through considerations of histidase activity, renal clearance, and carnosine metabolism.

Cardio-vascular effects of procaine amide. PEGGY JO NEWMAN* AND BYRON B. CLARK. *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

Procaine amide (Pronestyl-Squibb) is related chemically and pharmacologically to procaine with an amide substituted for an ester linkage between DEAE and PABA. The mechanisms of cardio-vascular action, on which the clinical uses of procaine amide depend, have been studied and the results reported herein. In anesthetized cats

and dogs, doses of procaine amide less than 10 mg/kg. produce no change in blood pressure; at 10 mg/kg., pressure usually falls slightly, while doses greater than 20 mg/kg. invariably produce hypotension. Procaine amide has one-fifth the vasodilator activity of procaine and papaverine as measured by femoral flow. EKG changes in anesthetized dogs and cats, in doses greater than 10 mg/kg., consist of bradycardia (without other changes in rhythm), depression and notching of T waves and QRS complexes and prolongation of PR and QRS intervals. In the exposed dog heart, with rate kept constant and intact innervation, conduction is slowed and auricular threshold elevated with 10–20 mg/kg. doses. In the frog ventricle strip exposed to procaine amide (1 mg/ml.) for 25 minutes conduction is slowed 45% while threshold is elevated 100%. In the isolated rabbit heart procaine and quinidine elevate ventricular threshold more but have a shorter duration than procaine amide. Contractility is equally depressed by procaine and quinidine, but unaffected, slightly increased or decreased by procaine amide which produces a proportionally greater elevation of threshold with less effect on contractility. Heart rate is uniformly slowed. The maximum driving rate in the isolated rabbit auricle is elevated equally by procaine and quinidine, each about six times more potent than procaine amide.

Blockade of responses to epinephrine and norepinephrine by Dibenamine congeners. MARK NICKERSON, JAMES W. HENRY* AND GEORGE M. NOMAGUCHI.* *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.*

The pressor response of the intact cardiovascular system to epinephrine is more readily 'reversed' by various adrenergic blocking agents than is the response to norepinephrine. Without allowance for the complexity of the cardiovascular system, this observation frequently has been interpreted as indicating that the effects of norepinephrine are less readily inhibited. In the case of blockade by the β-haloalkylamines, which appear to be firmly attached to specific 'receptors', this interpretation would imply that epinephrine and norepinephrine activate different receptors or that the chain of events between combination with the receptor and final contraction of the smooth muscle cell is quantitatively dependent upon the chemical configuration of the mediator. Detailed quantitative studies of the blockade of essentially pure excitatory responses to epinephrine and norepinephrine in cats by G-D 228 and SKF 688A (very potent phenoxyethyl derivatives of Dibenamine) were carried out on the chronically denervated nictitating membrane and on the intact cardiovascular system modified by inducing maximal adrenergic vasodilation and cardioacceleration with a continuous infusion of isopropylnorepinephrine. Tlockade of responses to epinephrine and norepinephrine was found to be qualitatively and quantitatively similar. These observations indicate that differences in the gross blood pressure response to various sympathomimetics after the administration of adrenergic blocking agents are due to disparities in their vasodilating and cardiac stimulating properties, actions which cannot be inhibited by any of the currently employed adrenergic blocking agents, rather than to any inequalities in the blockade of vasoconstriction.

Inside the cell. Part II: The regulation of enzyme activity (motion picture). ALEX B. NOVIKOFF AND JEROME A. SCHACK (introduced by I. NEUWIRTH). *Univ. of Vermont, College of Medicine, Burlington, and Beth Israel Hospital, New York City.*

Like Part I (*Federation Proc.* 8: 120, 1949), this film has been produced by the U. S. Army Med. Illustration Service, under the supervision of the Surgeon General. It attempts to show the importance to chemotherapy of a knowledge of the factors regulating enzyme action. Among the regulating factors discussed are the intracellular location of enzymes, changes in their concentrations resulting from changed functional and hormonal states, pH, concentrations of reaction products, metallic activators and inhibitors, and anti-metabolites. The enzymatic basis of drug action is shown by animation of the sulfanilamide-*p*-aminobenzoic acid and of the eserine-acetylcholine esterase relationships. Five investigators are shown in their laboratories: Dr. Walter C. Schneider, demonstrating the use of differential centrifugation to localize enzymes inside the cell; Dr. A. Szént-Györgi, showing the effect of adenosine triphosphate on isolated muscle fibers and actomyosin threads; Dr. Harland G. Wood, administering carboxyl C^{14}-labeled acetate to rats and demonstrating the isotope distribution in the glycogen molecule; Dr. D. W. Wooley, demonstrating mice in which thiamin deficiency has been produced by feeding the anti-vitamin, pyrithiamin; and Dr. David Nachmansohn, demonstrating the action of eserine on the conduction of impulses by the isolated nerve fiber of the squid. Extensively animated sequences include the path taken by C^{14}-labeled acetate in carbohydrate intermediary metabolism and the ionic, electrical and enzymatic changes which may occur during the transmission of impulses by nerves.

Action of rat liver slices upon pamaquine hydroiodide. SHIRLEY J. NYDEN (introduced by E.M.K. GEILING). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Recent evidence that the metabolites of pama-

quine are excreted in the urine of animals administered the drug suggested that the compound may be altered by the action of the tissues. The incubation of panaquine with rat liver slices resulted in a decrease in the concentration of the added drug when it was determined using the colorimetric technique of Brodie *et. al.* (*J. Biol. Chem.* 168: 32, 1947). Slices were placed in closed vessels containing oxygenated Krebs'-Ringer-Phosphate solution to which were added 0.04 M glucose and pamaquine in the concentration of 10^{-4} M. The vessels were incubated for 2 hours at 37.8° C. with shaking. A decrease of 40.2% was observed in the concentration of pamaquine remaining in the total system when compared to that measured in the control vessels incubated with boiled liver slices. The amount of the drug absorbed by the boiled tissue was estimated by comparison with controls incubated without tissue. The results obtained suggest that pamaquine is converted, *in vitro*, by respiring liver slices to metabolites not measured by the Brodie technique.

Comparative toxicity of halogenated hydrocarbons proposed for fire extinguishants. FRED W. OBERST, CHARLES C. COMSTOCK* AND FRANCIS P. MCGRATH.* *Med. Div., Army Chemical Center, Md.*

A series of 12 halogenated hydrocarbons and CO_2 either proposed or now in use as fire extinguishers were tested for inhalation toxicity in rats exposed 15 minutes. Each of these compounds was also subjected to pyrolysis by passing the vapor over an iron surface at 800° C. and the resulting mixture tested for inhalation toxicity. The method used by Deichmann and LeBlanc for determining approximate lethal dose of compounds administered by injection was adapted to the determination of inhalation toxicity of vapors dispersed in air. Exposures were made in a dynamic air flow chamber on one animal at a time in steps of increasing concentrations. Each step was 50% higher than the preceding one until a level was reached at which the animal died either during the exposure or during the 14-day observation period. The validity of this method was verified on methyl bromide by comparing the results with those from the LC50 method, using 10 animals at each level. The following compounds are in order of increasing toxicity: CF_3Br, CF_4, CF_2BrCl, $C_2F_4Br_2$, CO_2, C_2H_5Br, CF_2Br_2, CH_2ClBr, $C_2F_3ClBr_2$, $C_2H_2F_2Br_2$, CCl_4, CH_3Br and CH_3I. The ALC (approximate lethal concentration) for the least toxic compound is 5070 mg/1. or 834,000 ppm and for the most toxic is 22 mg/1. or 4000 ppm. The inhalation toxicity of nine of these compounds is increased on pyrolysis. Vapors of CH_3Br and CH_3I are less toxic after pyrolysis. Heating did not affect the toxicities of CF_4 and CO_2 due to their thermal stability. All of the compounds produced irritation to the respiratory system and varying degrees of narcosis.

Spasmolytic activity of phenyl γ-(2-pyridyl) propyl ketone (EJA II) and some related compounds. J. F. O'LEARY AND I. H. SLATER (introduced by HERBERT B. STOKINGER). *Div. of Pharmacology and Orthopaedic Surgery, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

It has been shown that many 2-substituted pyridyl and quinolyl compounds related to EJA II are potent central nervous system depressants. In anesthetized and in spinal cats, EJA II causes a transient fall in blood pressure. Neither atropine (0.1 mg/kg.) nor phenindamin (Thephorin, 4 mg/kg.) altered this response. EJA II did not affect the change in blood pressure noted after acetylcholine, epinephrine, or histamine. On isolated strips of rabbit ileum, EJA II and several related compounds in dilutions of 1:10,000–1:25,000 caused a decrease in tone and some depression of spontaneous contractility. When tested by the quantitative technique of Miller, Tainter and Becker (*J. Pharmacol. & Exper. Therap.* 92: 260–268), these compounds appear to be 1/5,000 as potent as atropine in antagonizing the spasmogenic activity of acetylcholine. On the other hand, EJA II, phenyl γ-(2-quinolyl) propyl ketone, phenyl γ-(2-pyridyl) α-phenyl propyl ketone and several related compounds appear to be almost as potent as papaverine as an antagonist to the action of barium chloride. After acetylcholine induced spasm, these compounds caused striking reduction in the motility as well as of the tone of the small intestine. On the other hand, the relaxation of barium induced contractures occurred without any decrease in intestinal motility.

A critique of laboratory methods for the evaluation of agents for use in collagen diseases. GERALD Q. O'NEILL, ROBERT T. FORD, DOROTHY N. DUGHI, ELAINE FADDIS, ROBERT L. PEIFFER AND WILLIAM S. WALLER (introduced by KARL H. BEYER). *Med. Research Div., Sharp and Dohme, Inc., Glenolden, Pa.*

In this laboratory the following four methods have been modified and adapted to the evaluation of agents for use in the treatment of collagen diseases: *1*) antihyaluronidase activity in mice, (OPSAHL. *Yale J. Biol. & Med.* 21: 487, 1949) in which the inhibition of the spreading effect of hyaluronidase in the skin of the mouse was measured; *2*) experimental plantar arthritis in the rat (SELYE. *Brit. M. J.* 2: 1129, 1949) in which the power of the agent to inhibit the production of arthritic and periarthritic changes in the foot of the rat was meaured. The degree of inhibition was determined by the change in the size of the affected foot, obtained by the displacement of mercury in a calibrated tube; *3*) functional activity of the

pituitary-adrenal system as revealed by the adrenal ascorbic acid depletion (SAYERS, SAYERS AND WOODBURY. *Endocrinology* 42: 379, 1948). Some indication of the mode of action of an agent as it relates to the pituitary-adrenal system has been obtained from the effect of the agent on the ascorbic acid level in the adrenal gland of the rat; 4) inhibition of the anaphylactoid reaction to egg white in the rat (SELYE. *Canad. M. A. J.* 61: 553, 1949). The overall interpretation of efficacy as measured by this battery of tests is in reasonable agreement with the general clinical impression of utility of such agents as cortisone, ACTH, Artisone, pregnenolone, testosterone, progesterone, hydroxyphenyl cinchoninic acid, desoxycorticosterone and related compounds.

Anticonvulsant and toxic effects of alpha-phenyl-butyrylurea, an aliphatic analogue of phenobarbital and phenurone. MARSHALL J. ORLOFF,* P. E. FELDMAN,* CHARLES H. SHAIOVA* AND CARL C. PFEIFFER. *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, and Manteno State Hospital.*

Alpha-phenyl-butyryl urea (PBU), the ethyl analogue of 'Phenurone' and the aliphatic analogue of phenobarbital, was found to be a potent anticonvulsant against artifically-induced convulsions in laboratory animals. Further, it was found to be chronically non-toxic in effective doses. In a group of 10 institutionalized patients with severe epilepsy, this drug appeared to be effective in controlling grand mal seizures. However, the drug produced severe toxic effects when doses necessary to bring about adequate control were given. PBU was not given an adequate trial in psychomotor epilepsy, but from the evidence now available it appears that the drug increases the frequency of psychomotor attacks. PBU was not tested in petit mal epilepsy. The extreme toxicity of alpha-phenyl-butyryl urea at effective anticonvulsant dosage levels precludes its use as a therapeutic agent in the treatment of epilepsy.

A clinical evaluation of the use of atropine or scopolamine alone as premedicating agents. O. SIDNEY ORTH, JOSELYN ROBB* AND K. E. LEMMER.* *Depts. of Pharmacology, Anesthesiology and Surgery, Univ. of Wisconsin Med. School, Madison.*

Scopolamine has come to be used rather extensively in anesthetic premedication by virtue of the psychic sedation in addition to salivary inhibition it produces. Frequently when used alone there is the complaint from surgeons or the nursing staff that the postoperative period of the patients is disturbed. Other factors could also be responsible. In an attempt to separate the drug effect as much as possible, 12 ambulatory patients over a week postoperatively were tested in random order at one- or 2-day intervals with the two belladonna derivatives administered intravenously in dosages of 0.3–0.6 mg., in the amount which would have been judged adequate for operative medication. Respiratory effects were determined from basal metabolism records, salivary flow was measured, and general attitude and behavior evaluated objectively. Salivary inhibition occurred within 5 minutes of drug administration but the effect with scopolamine definitely was more prolonged, usually lasting in excess of 2 hours. There was definite psychic sedation in 11 patients after scopolamine and in none after atropine. None of the patients were restless or exhibited mental changes after atropine while 5 with scopolamine were restless and 3 were mentally confused, in one of whom the period persisted into the second day. The administration of appropriate doses of morphine or other analgesic and depressant drugs before the patient recovers from the anesthetic depression counteracts such undesirable reactions.

Effect of stable and radioactive gallium on Q_{O_2} of rat liver slices. JESSE D. PERKINSON, JR. AND ROBERT S. INGOLS (introduced by H. D. BRUNER). *Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn., and Georgia Inst. of Technology.*

As the first in a series of studies of the effect of internal irradiation, the Q_{O_2} of liver slices was determined for rats sacrificed at 6, 12, 18, 24, 48, 72, and 96 hours after radioactive gallium plus stable gallium or an equivalent amount of stable gallium was injected by tail vein. By 6 hours, the Q_{O_2} was significantly depressed in the irradiated tissues and the same degree of depression was apparent at 12 hours. The Q_{O_2} values were normal during the 48- through 96-hour period. The effect of gallium irradiation on glycogen and water content was also determined.

Suppression of tachycardia following adrenergic blockade. ALBERT J. PLUMMER, WILLIAM B. REITZE* AND FREDRICK F. YONKMAN. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Adrenergic blockade following the use of Regitine (0.5 mg/kg.) (2-[N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl]-imidazoline) is uniformly accompanied by hypotension in the dog anesthetized with pentobarbital and commonly but not not uniformly by an associated tachycardia; varying degrees of tachycardia usually occur in man. Various measures have been employed to suppress the increase in heart rate while the hypotensive effect of Regitine is maintained. It has been observed that the incidence of tachycardia is much lessened when the absorption is slowed by using the subcutaneous or intestinal route of administration. After tachycardia has fully de-

veloped following intravenous administration of Regitine however, 5–30 μg/kg. of veratramine intravenously or 30–100 μg/kg. introduced into the intestine of the dog almost completely negate the cardioaccelerator effect. Since veratramine prevents the cardiac speeding after epinephrine it is possible that its action in this case might be directed against "spared". circulating epinephrine which is not metabolized due to adrenergic blockade of receptors at adrenergic effector sites elsewhere. The 'antiaccelerating effects' (Krayer) of veratramine and other veratrum principles on tachycardia produced by other adrenergic blockers will also be presented.

Action of polypropylene glycols with average molecular weights of 400, 750, 1200 and 2000 on the cardiovascular and central nervous systems of the dog. LEONARD PROCITA* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

It has been reported previously (*Federation Proc.* 8: 332, 1949) that polypropylene glycol 750 is capable of producing a bigeminal rhythm of ventricular origin in the anesthetized dog and this action is in some way related to a relative anoxia of the myocardium. This property is shared by polypropylene glycol 400 but not by polypropylene glycols 1200 or 2000. Similarly, the two lower molecular weight substances produce a pressor response but the other two do not. This pressor response is eliminated by the exclusion of the adrenals from the circulation, but is not abolished by tetraethylammonium chloride. This latter observation suggests that the pressor response is due to a direct action on the adrenal medulla. The fact that the two higher molecular weight substances neither possess this action nor produce the ventricular arrhythmia suggests that the bigeminal rhythm which appears following the administration of the two lower molecular weight compounds is dependent upon the existence of not only a relative myocardial anoxia but also an increase in circulating epinephrine or sympathin. The polypropylene glycols are also potent central nervous system stimulants. Electroencephalographic studies show they possess the unusual property of producing a spreading type of increased electrical activity in the cortex. The marked antagonism of polypropylene glycol 750 to mephenesin depression of the flexor reflex in the decerebrate dog indicates the excitant activity is present at midbrain and lower levels.

Subacute oral toxicity of ipecac and the accumulation of its alkaloids in tissues. JACK L. RADOMSKI,* ERNEST C. HAGAN,* HENRY N. FUYAT* AND ARTHUR A. NELSON. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Little attention has been given to the possibility of absorption and accumulation of harmful levels of the ipecac alkaloids (largely emetine and cephaeline) after the oral ingestion of crude ipecac. Ipecac has been proposed for inclusion in oral barbiturate preparations to prevent death from overdosages. Experiments conducted on dogs and cats showed that ipecac is ineffective in preventing anesthesia and death from large doses of pentobarbital. The subacute toxicity of ipecac to rats, cats, and dogs was investigated. A dietary level of 1000 ppm was fatal to rats in 7–26 weeks. Daily doses as low as 62.5 mg/kg. were fatal to 2 of 3 cats after 5 and 13 doses. Daily doses as low as 20 mg/kg. killed 3 of 4 dogs in 3–4 weeks, and the fourth after 61 weeks. Histopathological examination revealed some liver damage. The tissues of certain of these animals were examined chemically for ipecac alkaloids by Gimble's ultraviolet absorption method and Gimble's modification of Brodie's methyl orange method (*J. Pharmacol.* 94: 431, 1948). The livers of rats which died on 1000 ppm of ipecac showed levels up to 14 mg. of ipecac alkaloids per 100 grams. The livers of dogs receiving 20 mg/kg. showed the presence of 1.2–4.9 mg/100 gm. This represents approximately 10–30% of the total alkaloids administered.

A quantitative method for determining the ability of surviving renal slices to concentrate phenolsulfonphthalein. ROBERT C. RATHBUN* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Quantitative data on phenolsulfonphthalein (PSP) concentration by slices of surviving kidney cortex have been obtained in the past by varying the concentrations of PSP, substrates or inhibitors and noting the ratio of concentrations of any two of these three variables required to produce an all or none effect, i.e. visible concentration or no concentration. Considerably more information could be acquired with less effort if it were possible to quantitatively determine the concentration of PSP in the slice. Slices of guinea pig renal cortex were incubated in a medium as described by Beyer *et al.* (*Am. J. Physiol.* 161: 259, 1950), removed after incubation, blotted and weighed. The slices were extracted for one hour with water in a continuous extractor, the extract evaporated to dryness and the residue taken up in 0.2 ml. of water. To this, sufficient ethyl alcohol (95%) was added to make a final volume of 10 ml. The solution was centrifuged to remove any insoluble material, an aliquot evaporated to dryness, the residue dissolved in 0.1 N sodium hydroxide and the concentration of PSP determined spectrophotometrically. Employing this method it has been found that *1*) PSP concentration in the slice begins within one minute and reaches a maximum

in 15 minutes, *2*) maximum concentration is affected by the ratio of slice weight to medium volume, and *3*) if concentration is partially inhibited by dehydroacetic acid reversal is afforded by sufficiently high concentrations of sodium acetate.

Influence of respiratory acidosis on the plasma levels of thiopental sodium. CHALMERS J. RAYBURN,* RICHARD W. WHITEHEAD AND WILLIAM B. DRAPER. *Dept. of Physiology and Pharmacology, Univ. of Colorado Med. Center, Denver.*

The effect of respiratory acidosis on the plasma levels of thiopental (Pentothal) sodium in dogs has been studied. The control animals were given pure oxygen, the experimental animals either 10, 20, 30, or 40% carbon dioxide in oxygen or were under the influence of endogenous carbon dioxide which accumulated during diffusion respiration. It was found that with the establishment of respiratory acidosis—the pH of venous blood ranging from 7.25–6.80—the plasma level of thiopental decreased by as much as 50%, even though the intravenous injection of the anesthetic agent was continued during this time. Upon the withdrawal of carbon dioxide, followed by the administration of oxygen, the plasma level of thiopental rose concurrently with blood pH, even in those animals which had received no additional thiopental sodium for periods of 30–60 minutes. After the fifteenth minute of diffusion respiration the animals showed the same pattern of distribution of thiopental sodium as that of the dogs which had inhaled carbon dioxide. Blood volume changes have been studied under the above conditions and will be discussed in relation to the plasma thiopental changes observed. (Supported by a grant from the Office of Naval Research.)

Isolation of 17-hydroxycorticosterone and other steroid ketones from adrenal venous blood of cows. HANS REICH, DON H. NELSON AND FRANK X. GASSNER (introduced by LEO T. SAMUELS). *Dept. of Biochemistry, Univ. of Utah College of Medicine, Salt Lake City, and Exper. Station, Colorado A and M College, Fort Collins.*

By means of a special catheter, adrenal venous blood was collected from cows before and after administration of ACTH. The blood was then examined for adrenocortical steroids. Several distributions between dilute alcohol and hexane were carried out, followed by separation of the ketones with Girard's reagent and subsequent acetylation. The crude acetylated product showed strong absorption at 240 mμ, but only a small amount of 17-hydroxycorticosterone acetate (Kendall's Cmpd. F acetate) could be isolated by chromatography. At least two other compounds were found which also showed absorption at 240 mμ. One of these substances melted at 205–210° and probably contained two keto groups, one hydroxyl group and one acetoxy group, as shown by the infrared spectrum. The other substance did not crystallize thus far. It gave a positive Zimmermann reaction typical for 17-ketosteroids and showed peaks at 5.70 μ and 5.93 in the infrared. Furthermore, the presence of one hydroxyl and one acetoxy group could be established. Surprisingly enough only the 17-keto group reacted with semicarbazide acetate in alcohol-pyridine. This makes it highly improbable that the α,β-unsaturated keto group is in 3-position. After oxidation with chromic acid the infrared spectrum showed three keto groups, the newly formed one probably in 11-position. There is good evidence that this C_{19}-ketone is closely related to the androgen which was found to be present in the feces of pregnant cows (LONGWELL AND GASSNER. *Federation Proc.* 6: 272, 1947).

Effect on veratrine alkaloids on the carotid sinus pressor reflex. ARTHUR P. RICHARDSON, CAROLYN FARRAR* AND HARRY A. WALKER. *Dept. of Pharmacology, Emory Univ. School of Medicine, Emory University, Ga.*

It has long been recognized that veratrine alkaloids may induce a bradycardia, fall in blood pressure, and depression of respiration, by virtue of reflex stimulation on the vagus nerves. This reaction, however, does not account for the total hypotensive action of these agents, since they still produce a marked fall in pressure after vagotomy. The mechanism of this latter reaction has remained obscure. We have found that many veratrine alkaloids possess the property of depressing and blocking the carotid sinus pressor reflex, at a time when blood pressure has fallen very little. The following preparations have been assayed by this procedure and the dose (mg/kg.) required to produce a 50% depression of the carotid reflex are as follows: germitrine .0013, protoveratrine .003, germine >10.0, germerine .003, germidine .003, Veriloid .008, veratramine >0.3 and jervine >1.0. The mechanism of this effect will be discussed.

Cyanide in the blood of dog and man following the administration of thiocyanate. FREDRIC RIEDERS AND FRANZ GOLDSTEIN (introduced by CHARLES M. GRUBER). *Depts. of Pharmacology and Surgical Research, Jefferson Med. College, Philadelphia, Pa.*

Goldstein and Holburn (*J. Pharmacol. & Exper. Therap.* 96: 285, 1949) have shown that poisoning of the dog with thiocyanate resulted in histotoxic hypoxia. Following the intravenous injection of NaSCN (300 mg/kg.) increasing amounts of cyanide determined by the method of Gettler and Goldbaum (*Anal. Chem.* 19: 270, 1947) and decreasing amounts of SCN^- were found in the blood of 5 dogs (average CN^- mg/100 ml. blood cells: 0.115 after 2 hours; 0.215 after 4 hours; 0.325 after 6 hours; 0.400 after 8 hours; 0.480 after 10 hours).

Several hours following single injections of about 700 mg. NaSCN for the determination of extracellular fluid space cyanide could be detected in the blood of eight patients (0.005–0.05 mg/100 ml. blood cells). Most of the cyanide was found in the cellular fraction of the blood. The significance of these experimental findings in relation to the toxic and hypotensive action of thiocyanate will be discussed.

Effects of cortisone on intradermal pneumococcal infections in rabbits. HARRY J. ROBINSON. *Merck Inst. for Therapeutic Research, Rahway, N. J.*

The effect of cortisone on the course of an infection produced by the intradermal injection of virulent Type I pneumococci was studied in rabbits. Cortisone was given subcutaneously in doses of 1–3 mg/kg. animal body weight over a 7-day period prior to the pneumococcal injection and thereafter until the experiment was terminated. Under these conditions, cortisone lowered the resistance of rabbits to this infection as judged by the dermal lesion, erythrocyte sedimentation rate, blood culture, mortality and histopathology. Dermal lesions in non-cortisone treated rabbits were relatively well localized measuring approximately 3–4 square inches at the 48-hour observation period. In contrast, the dermal lesions in cortisone treated rabbits measured approximately 28 square inches. Blood cultures revealed the early appearance of a pneumococcal bacteremia in the cortisone treated animals leading in most instances to death of the host within 4–10 days. Non-cortisone treated rabbits usually developed a transient low grade bacteremia and most animals survived the infection. Other studies including the histopathology, blood picture, erythrocyte sedimentation rate, also indicated that the disease was more fulminating in rabbits receiving cortisone. Penicillin was able to control the infection in both cortisone and non-cortisone treated animals although a greater dose of penicillin was required when cortisone was administered.

A method of testing for vasoactivity in the isolated guinea pig kidney using a constant flow perfusion system. R. R. ROEPKE AND G. H. BREWER (introduced by J. T. LITCHFIELD, JR.). *Stamford Research Labs., American Cyanamid Company, Stamford, Conn.*

A constant flow perfusion system described elsewhere was used to study the vasoactivity of the isolated guinea pig kidney. A balanced salt solution was perfused through the kidney via the renal artery, the pressure in the perfusion line adjacent to the renal cannula being recorded and taken as a measure of the peripheral resistance of the kidney. An investigation was made of the conditions affecting the reactivity of the renal vessels, as shown by responses to the reference drugs epinephrine and acetylcholine or papaverine. The temperature as well as the composition of the perfusion fluid was found to be important in the maintenance of sufficient vascular tone to give reversible responses to vasodilating drugs. With usable preparations of kidneys, effective doses of the drugs in micrograms are of the order of 0.02 for l-epinephrine, 0.002 for acetylcholine and 0.3 for papaverine with a gradual decrease in sensitivity with time. Most usable preparations gave satisfactory responses for 2–3 hours with some lasting 5–6 hours.

Experimental diabetes produced by 8-hydroxyquinoline. MARY A. ROOT* AND K. K. CHEN. *Lilly Research Labs., Indianapolis, Ind.*

It was recently reported by Kadota (*J. Lab. & Clin. Med.* 35: 568, 1950) that intravenous administration of 8-hydroxyquinoline (oxine) to rabbits produced an alloxan type of diabetes. In repeating some of this work we found that the production of a transient hyperglycemia occurred in all our rabbits but that a persistent hyperglycemia resulted in only 9 of the 29 rabbits which were injected intravenously with 8-hydroxyquinoline. The duration of hyperglycemia in these 9 rabbits varied from 4–16 days. When methylene blue (50 mg/kg.) was injected intravenously immediately before the administration of 8-hydroxyquinoline, hyperglycemia of from 14 to more than 40 days duration occurred in 8 of the 14 rabbits so treated. Apparently methylene blue potentiates the diabetogenic action of 8-hydroxyquinoline just as it does with alloxan, as was recently reported by Lazarow (*Proc. Soc. Exper. Biol. & Med.* 73: 323, 1950). In rabbits the diabetogenic dose of 8-hydroxyquinoline (50 mg/kg.) was close to the lethal dose (65–75 mg/kg.). Death was caused by respiratory failure. If artificial respiration was applied immediately after injection of the drug, death could usually be prevented. A moderate degree of methemoglobinemia was produced in rabbits, cats, and dogs whenever 8-hydroxyquinoline was administered intravenously. In rats preliminary experiments with intravenous administration of 8-hydroxyquinoline did not produce even a transient hyperglycemia.

Reversible agglutination of trypsin modified erythrocytes by normal human sera. MARTIN ROSENTHAL AND LAWRENCE SCHWARTZ (introduced by JOSEPH H. PRATT). *Ziskind Labs., J. H. Pratt and New England Center Hosps. and Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Human erythrocytes treated with bacterial filtrates, certain viruses, and periodate ions may be agglutinated by normal human sera. We have observed that trypsin modified cells are similarly agglutinated by sera, free from any of the usual complete or incomplete hemagglutinins. Aggluti-

nation titers ranged from 1–32. The responsible agglutinin displayed activity against auto- and iso-cells. Immediate centrifugation of the trypsin modified erythrocyte-serum-saline mixtures was necessary else the agglutination effect was lost. Agglutinates, once formed, broke up on continued contact with serum (agglutination reversal effect); this was not due to loss of the agglutinin, since the serum-saline dilutions still displayed activity against fresh lots of trypsin modified erythrocytes. Rather, the erythrocytes incubated with serum underwent alterations making them resistant to the action of the agglutinin. Warmth hastened and cold inhibited this erythrocyte alteration. These two effects of serum, agglutination and agglutination reversal, could be readily dissociated since the agglutinin was non-dialyzable, thermostabile, and sensitive to pH changes, while the agglutination reversal effect was lost on dialysis, and was resistant to heat and pH changes. Exposure of erythrocytes *in vivo* to the action of proteolytic-enzymes derived from tissue breakdown may render them susceptible to the agglutinin described above. Intravascular agglutination, with either massive clumping or hemolysis might follow. The serum factor responsible for the agglutination reversal effect would have a protective, hemeostatic function under these circumstances.

Traumatic shock in mice. Hemodynamic effects of therapy and their correlation with mortality. SANFORD M. ROSENTHAL AND R. CARL MILLICAN.* *Natl. Insts. of Health, Bethesda, Md.*

A standardized burn was employed, 95–100% fatal in untreated mice. Therapy consisted of 1 ml/20 gm. mouse of the following: (intravenous) mouse whole blood or plasma, human serum albumin in glucose or in 0.9% NaCl, or saline alone. One group received saline orally; controls received no treatment. Bleeding volume, hematocrit, plasma proteins, and rectal temperature were determined at ¼, 2, 4, 6½ and 22 hours after therapy. The following results were obtained. At ¼ hours bleeding volume was restored to values higher than normal by whole blood; to normal values by plasma, or albumin in saline or glucose; to about half normal by saline i.v., and only slightly by saline orally. Corresponding effects on hematocrit occurred, with the exception of whole blood therapy. Plasma proteins from ¼–6½ hours were above normal in all groups except saline i.v. or oral, where decreases occurred throughout the experiment. Within 2 hours after therapy the effects of plasma and albumin on bleeding volume and hematocrit had decreased to the point where no significant differences between electrolyte and colloid therapy were present. With whole blood the bleeding volume remained slightly higher and hematocrits remained near 85% for more than 7 hours. Albumin in glucose approached values of untreated controls. Rectal temperatures fell progressively, except for transitory rises coinciding with bleeding volume increases. Mortality studies showed plasma, whole blood, saline i.v. and orally, and albumin in saline equally effective; albumin in glucose was without effect. Therefore, certain acute hemodynamic responses to therapy may be unreliable as criteria of therapeutic effectiveness.

Inotropic action of blood serum fractions upon the frog heart and cat myocardium. WILLIAM T. SALTER, NICHOLAS J. GIARMAN* AND JACK PETER GREEN.* *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*

The chemical fractions of E. J. Cohn from bovine serum and human serum have been tested *in vitro* on the isolated Straub frog heart and on the Gold-Cattell feline papillary muscle. The serum fractions were added to Krebs-Hensleit solution which was low in calcium. Appropriate compensation was made for the calcium-binding of the inherent protein. Whereas the addition of calcium caused an immediate inotropic response by the hypodynamic muscle, the serum fractions slowly increased the myocardial contractions over half an hour. This response, therefore, simulated that produced by adding digitoxin to a hypocalcic medium. The effect could be removed slowly by perfusing with fresh hypocalcic Krebs-Hensleit solution; and again the addition of serum fractions produced an inotropic effect. The albumin fractions appeared particularly effective; but it is not certain that the pharmacophor group is necessarily colloidal.

Effect of thyroidectomy and propylthiouracil upon the pyrogenic action of Dicumarol. LLOYD D. SEAGER AND PHILIP W. BERNSTORF.* *Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock.*

Normal dogs, rabbits, and rats show a marked hyperpyrexia to the oral or parenteral administration of a suspension of Dicumarol. Terminal temperatures of 110–112 are often observed. The animals present a picture similar to that of dinitro phenol poisoning. Survival time may be shortened to 1 hour in rats with doses of 500–700 mg/kg. intraperitoneally and to 2–4 hours in rabbits with similar doses. Thyroidectomized rats and rabbits show diminished or no pyrogenic response. Thyroidectomized animals treated for 2–3 months with propylthiouracil show no pyrogenic response. The lethal effect of Dicumarol is not found to be diminished by thyroidectomy or propylthiouracil. This finding negates the theory that the acute lethal effect of Dicumarol is due to the hyperpyrexia. The role of the thyroid hormone in the pyrogenic action of Dicumarol appears to be similar

to that reported by Barker (*Federation Proc.* 5: 4, 1946) for the metabolic effects of dinitro phenol.

Effect of mercurials on rat tissue enzyme systems. R. A. SEIBERT* AND C. A. HANDLEY. *Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

Both inorganic and organic mercury containing compounds have been demonstrated to inhibit *in vitro* enzyme systems containing sulfhydryl groups essential to activity. Mercurial diuretics *in vivo* strongly inhibit the succinic dehydrogenase activity of rat and dog kidney homogenates, when the compound is administered intravenously in a non-toxic diuretic dose. This effect is probably selective on the kidney due to the rapid accumulation of mercury in this organ by intra-tubular reaction of mercurial and sulfhydryl groups. Even lethal concentrations of diuretics, that kill rats within one hour by ventricular fibrillation, fail to inhibit the cardiac succinic dehydrogenase system but a 15–20% inhibition of the liver and approximately a 50% inhibition of the kidney systems occur. Thus lethal concentrations of mercurial diuretics have little more depressant action on the kidney system than ordinary diuretic doses. The effects of diuretics on several other enzyme systems will be presented.

Effect of cortisone and other steroids on the hibernating gland of the pregnant white rat. JOSEPH SEIFTER, JOHN J. CHRISTIAN* AND WILLIAM E. EHRICH.* *Wyeth Inst. of Applied Biochemistry, Wyeth Inc.; Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

The effects of cortisone, pregnenolone, 21-acetoxypregnenolone, and desoxycorticosterone on the course of pregnancy were investigated. Weight changes and histological structure of the hibernating gland, the adrenals, and the pituitary, and incidence of resorption of fetuses were noted. Cortisone produced the most striking effect on the course of pregnancy. It caused an increase in the rate of fetal mortality probably due to changes in the ground substance of the placental attachment, without traversing the placental barrier. Pregnenolone produced changes in the fetal structures comparable to those in the maternal ones. The other steroids had questionable effects. Cortisone, DCA and pregnenolone altered the normal cyclic changes in the hibernating gland which occur during pregnancy. Cortisone exaggerated the increase in weight and decrease in density which normally begin at the time of implantation. This was reflected microscopically as increased fat accumulation. Pregnenolone abolished the initial weight loss which precedes implantation and increased the weight gain which occurs in the latter third of pregnancy. Pregnenolone and DCA caused a marked increase in specific gravity which was manifested macroscopically by increased coloration and microscopically by discharge of lipid droplets and alteration of the cells, which appeared to acquire the characteristics of resting adrenal fasciculata. In the adrenal, cortisone affected chiefly the inner fasciculata and 21-acetoxypregnenolone the glomerulosa. The above changes are related to changes in the adrenal and in the pituitary.

Local antihistaminic activity of antihistamines in the rabbit eye. M. SHAW,* J. GIBLIN,* M. T. SPOERLEIN,* S. MARGOLIN* AND R. TISLOW. *Biological Research Labs., Schering Corp., Bloomfield, N. J.*

A procedure is described for measuring the local antihistaminic activity of antihistamines in the rabbit eye. Trypan blue (2% solution) was injected intravenously at a dosage of 2.5 cc/kg. and at the injection rate of 2 cc/minute. Immediately following the injection of the dye, 4 drops of antihistamine solution were applied to one eye; the other eye received physiological saline control solution. After 3 minutes, both eyes were washed with saline, and 4 drops of an 0.01% solution of histamine dihydrochloride were applied. When histamine is applied to the untreated rabbit eye, the nictitating membrane and the eyelids turn a deep blue in approximately 6 minutes, whereas, local pretreatment with a suitable antihistamine delays the appearance of this color. The antihistamines were assayed for their ability to inhibit the increase in permeability to dye caused by the local application of histamine. At least four eyes were used for each concentration (0.05%, 0.1%, 0.25%, 0.5%) of drug. The average time interval between the application of the histamine and the appearance of the deep blue color was calculated, and a dose-time curve was obtained relating concentration of antihistamine to the time of appearance of dye.—Chlorprophenpyridamine maleate (Chlor-Trimeton Maleate) was the most active of the antihistamines tested, and prophenphyridamine (Trimeton) was also very effective in delaying the appearance of dye. Phenazoline HCl (Antistine), tripelennamine HCl (Pyribenzamine), and diphenhydramine HC. (Benadryl) had little or no inhibiting activity. Actually, phenazoline, tripelennamine, and diphenhydramine in higher concentrations (0.5%) accelerated the appearance of the dye to less than control values—suggesting that these antihistamines probably increased the permeability of the tissue to trypan blue.

Cholinesterase as a cholinergic blocking agent *II.* GUY C. SHEATZ,* ALEXANDER G. KARCZMAR* AND THEODORE KOPPANYI. *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

It has been shown (KOPPANYI AND KARCZMAR. *Anat. Record.* 101: 19, 1948, *and Federation Proc.* 8: 309, 1949) that purified true cholinesterase (Winthrop-Stearns) can be injected and that it inhibits the muscarinic and nicotinic effects of acetylcholine in dogs, cats and turtles but is less effective in preventing cardiac slowing due to faradic vagal stimulation. Additional data indicate ineffectiveness of injectable true cholinesterase against pressor effects due to nicotine or faradic stimulation of cervical sympathetic. To explain these results, cholinesterase activity was assayed manometrically in blood and autonomic ganglia of cats prior to and following the injection of Winthrop-Stearns' cholinesterase. 5000 units/kg. of the latter caused, within 5 minutes, a 100% rise of cholinesterase activity in plasma, but none in red blood cells and in ganglia. These results show that practically all injected cholinesterase can be recovered from the plasma alone. Within 20–30 minutes plasma cholinesterase activity returned to normal. The duration of the increased cholinesterase activity in the plasma can be correlated with the duration of the blocking effects of injected cholinesterase on acetylcholine (o.c.). *In vitro*, Winthrop-Stearns' cholinesterase remains active in blood for at least 48 hours. Its rapid disappearance from blood *in vivo* can be correlated with the earlier data (o.c.) showing that pharmacological effects of injected cholinesterase are prolonged on shunting off the liver. The present data indicate also that, on injection, true cholinesterase becomes part of 'transport' cholinesterase which terminates the effects of acetylcholine before it reaches the effectors; transport cholinesterase is not thus exclusively pseudocholinesterase.

Effects of strophanthin-g on coronary circulation in the dog. THEODORE R. SHERROD (introduced by CARL C. PFEIFFER). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.*

Strophanthin-g, in doses of 10 μg/kg. of body weight was administered intravenously to 7 anesthetized open-chest dogs under positive artificial respiration with oxygen. The coronary sinus was cannulated through the right auricle by means of a specially designed metal cannula. Heparin was employed as an anticoagulant and the coronary sinus outflow was measured kymographically by means of an electrically wired, calibrated tipping bucket. Following the second or third dose of strophanthin in every case there was a decrease in coronary sinus outflow, averaging 25% below control levels (ranging from −11 to −57%). Beginning about the fifth or sixth dose there was a rather marked increase in coronary sinus outflow which averaged 96% above control levels (ranging between +36 to +228%). These changes in coronary sinus outflow showed no definite correlation with changes in blood pressure or heart rate. The experiments usually terminated in ventricular fibrillation which occurred following the tenth or eleventh dose of strophanthin-g.

Fixation of radioactive digitoxin by isolated hearts. A. SJOERDSMA AND C. FISCHER (introduced by E. M. K. GEILING). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Isolated hearts of rats, guinea pigs, rabbits and cats were perfused by the Langendorff technique with 150 cc. of Ringer-Locke solution containing 0.2 μg. of radioactive digitoxin per cc. Successive 25 cc. samples of perfusate were collected, and the total carbon 14 and digitoxin in each were determined. The hearts were similarly analyzed for total fixation and percentage of unchanged digitoxin. All solutions were plated on copper disks and counted in an internal Geiger counter. Digitoxin was extracted by utilizing its differential adsorption on alumina, with 10% ethanol in chloroform as the eluent. The results in all of the species studied indicate a rapid initial uptake of digitoxin followed by a slow but persistent fixation throughout the course of the perfusion. There was a relatively constant fixation of radioactive digitoxin per gram of heart by rat and guinea pig hearts, while the uptake by rabbit and cat hearts showed considerable variation. Fifty to 60% of the total radioactivity could be extracted from the hearts as digitoxin. Experiments on the reversibility of digitoxin fixation indicate that the concentration of digitoxin and its metabilites in the heart can be lowered appreciably by washing, but with the exception of the rat a small quantity always remained. It was also found that digitoxin was less firmly fixed than its metabolites.

Procaine Ascorbate. DONALD SLAUGHTER AND ROBERT HAZEL.* *Dept. of Physiology and Pharmacology, Univ. of South Dakota, Vermillion.*

A chemical combination of ascorbic acid and procaine (Procaine Ascorbate) exhibits an LD_{50} of 1000 mg/kg. and 500 mg/kg. for rats and mice respectively as compared to 300 mg/kg. and 225 mg/kg. ± 20 mg. for procaine hydrochloride administered separately. Similarly, simple addition of ascorbic acid to procaine hydrochloride markedly reduces the toxicity of procaine. Contrary to Richard and Kueter (*Anesth. & Analg.* 22: 283, 1943), sodium bisulfite in dilute concentration did not significantly alter these findings. The low toxicity of Procaine Ascorbate would indicate it worthy of clinical trial.

Antagonistic action of N-allyl-normorphine upon the analgetic and toxic effects of morphine, methadone derivatives and isonipecaine. C. C. SMITH, E. G. LEHMAN AND J. L. GILFILLAN (in-

troduced by HANS MOLITOR). *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Unna (*J. Pharmacol. & Exper. Therap.* 79: 27, 1943) and Hart and McCawley (*J. Pharmacol. & Exper. Therap.* 82: 339, 1944) observed that N-allyl-normorphine (NANM) reduced or abolished certain actions of morphine. However, Unna found NANM to possess only weak analgetic activity in mice whereas Hart and McCawley reported the compound to be as potent as morphine in rats. In the present study the analgetic potency of NANM has been reinvestigated in dogs and rats, and the antagonism between NANM and several analgesics has been examined. NANM was found to have a weak analgetic action in rats. In dogs, doses of 2–30 mg/kg. had no effect on the pain threshold. In rats, s.c. injection of 10 mg/kg. NANM prevented the rise in pain threshold, respiratory depression and catalepsy obtained by injecting 6 mg/kg. morphine or 2 mg/kg. of methadone, isomethadone, heptazone, or acetyl-dl-methadol. NANM only partially inhibited the analgetic effects of isonipecaine. When NANM was injected in dogs one hour after the administration of small active doses of morphine, methadone, heptazone, acetyl-dl-methadol, or isonipecaine, the pain thresholds returned to control levels in 5–10 minutes and the dogs no longer exhibited symptoms of respiratory depression, bradycardia, hypothermia, and miosis. Similar effects were obtained in 1–3 minutes when the antagonist was given i.v. Small doses of NANM (10 mg/kg.) protected mice and rats against otherwise fatal doses of methadone, isomethadone, heptazone, and acetyl-dl-methadol.

Excretion of gentisic acid in man as influenced by alkalinization and acidification. EVELYN M. SOMMER* AND ROBERT C. BATTERMAN. *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.*

The urinary excretion of gentisic acid in free and combined forms when administered orally as sodium gentisate was studied in man. When equilibrium of excretion was attained after 7 days of administration of the drug, analysis was done on 3 successive days. In 9 subjects, the daily recovery of gentisic acid averaged 62.4% and 26.3% in combined and free forms respectively. The percentage of the total gentisic acid excreted in the free form averaged 41.7%. Alkalinization with simultaneous administration of sodium bicarbonate in 6 subjects resulted in an increase of total and free gentisic acid excreted. The percentage increase averaged 35.3% and 51.4% for combined and free gentisic acid respectively. Acidification with simultaneous administration of dilute hydrochloric acid in 6 subjects resulted in a decrease of gentisic acid averaging 17% and 4.6% for combined and free respectively. Comparative studies with salicylates indicated that the gentisic acid excreted as an integral part of salicylate degradation was also influenced as above by simultaneous alkalinization and acidification.

A new repository penicillin. J. N. SPENCER, H. G. PAYNE AND V. YOUNG (introduced by F. H. SCHULTZ, JR.). *Pharmaceutical Research Div., Commercial Solvents Corp., Terre Haute, Ind.*

A number of salts of penicillin G have been prepared and compared with procaine penicillin as regards toxicity and duration of penicillin plasma levels. One of these, the penicillin salt of 1,2-diphenyl-2-methylaminoethanol in aqueous suspension, gave penicillin plasma levels lasting 24 hours or longer in 17 of 18 dogs, while none of 6 dogs treated with procaine penicillin had plasma levels lasting 24 hours. This new salt has a theoretical potency of 1050 units/mg. and is stable for 96 hours at 100° C. The acute intraperitoneal toxicity in mice of this new penicillin salt does not differ significantly from that of procaine penicillin. Likewise, there is no significant difference in the acute subcutaneous toxicity of the new salt and procaine penicillin in mice, rats and dogs. Mice have been administered daily subcutaneous doses of the new salt and procaine penicillin of 625, 1,250 and 2,500 mg/kg. for 14 days. The only deaths occurred at the 2,500 mg/kg. dose, one of 5 mice receiving procaine penicillin surviving and none of those receiving the new penicillin salt. Tissue sections prepared from these animals showed no pathology other than chronic irritation at the injection site. There was no detectable difference, however, in the degree of irritation produced by procaine penicillin and that produced by the new penicillin salt. Results of chronic studies with the new penicillin salt in rats and dogs will be reported.

Complete protection against lethal anaphylactic shock in guinea pigs by oral Chlor-Trimeton Maleate. M. T. SPOERLEIN,* E. MAKOVSKY,* S. MARGOLIN* AND R. TISLOW. *Biological Research Labs., Schering Corp., Bloomfield, N. J.*

According to the literature most antihistamines, even at relatively high dosage levels, afford only partial protection against anaphylactic shock in animals. In the present investigation, it has been found that small doses of oral chloroprophenpyridamine maleate (Chlor-Trimeton Maleate) completely protect sensitized guinea pigs challenged with 100% lethal doses of antigen. Several lots of guinea pigs weighing 200–400 gm. were sensitized by two intra-peritoneal injections (0.5 cc. each) of horse serum at an interval of 48 hours. Fourteen days after the initial injection of horse serum, graded doses of the antihistamine were fed. They were challenged exactly 3 hours after the drug

administration by the injection into the saphenous vein of 1.0 cc. of horse serum. The mortality within the first 10 minutes following the horse serum challenge was recorded, and the median protective dose (PD50) was calculated. Sensitized control guinea pigs were challenged with the lethal dose of horse serum (1.0 cc.) in each experimental series. The PD50 of chlorprophenpyridamine maleate was 0.370 mg/kg.; all animals survived at a level of 1.2 mg/kg. In a comparison of the protective effect of chlorprophenpyridamine maleate with tripelennamine HCl against lethal anaphylactic shock, the PD50 (mg/kg.) for chlorprophenpyridamine maleate was 0.375 and for tripelennamine HCl, 13.2.

Pharmacological actions of 'endothal' (disodium-3,6-endoxo-hexahydrophthalic acid). STANLEY E. SRENSEK AND GEOFFREY WOODARD (introduced by ARNOLD J. LEHMAN). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Endothal, closely related chemically to cantharidin, has been proposed as a selective defoliating herbicide. Intravenous injection of 5–10 mg/kg. in dogs resulted in an immediate scratching of the nose followed in 100 minutes by intermittent retching and vomiting persisting for 60–80 minutes, after which the animals either recovered or died of respiratory failure. Rabbits at 25–50 mg/kg. likewise pawed at their noses and shook their heads, then showed irregular breathing, and after 80–130 minutes, died of respiratory failure. Atropinized and non-atropinized dogs anesthetized with phenobarbital responded after 45–60 minutes to a 10 mg/kg. dose with a gradual decline in blood pressure until death supervened from respiratory failure. Electrocardiograms recorded simultaneously revealed no striking changes until after respiration ceased. Cats reacted similarly but with less regular decline in blood pressure. Immediately following respiratory failure, the diaphragm still responded to mechanical stimulation of the phrenic nerve. Domestic fowl were insensitive to doses four times as large as in the dog and cat. When moderate concentrations were used in isolated tissue experiments, no effects were noted on the rat phrenic nerve-diaphragm; preparations of rabbit and guinea pig small intestine showed a decrease in tone, but increased amplitude of rhythmic contractions; and dog carotid and calf aorta slowly increased in tone. Both *in vitro* and *in vivo* responses were characterized usually by a long delay following dosage.

Acute and chronic toxicity of Toluidine Blue and Neutral Red. FRANCES STOLARKSY AND THOMAS J. HALEY. *School of Medicine, Univ. of California, Los Angeles.*

Although Toluidine Blue is being used as a heparin inactivator, little is known concerning its acute and chronic toxicity. Furthermore, this dye is less potent than Neutral Red in counteracting heparin *in vitro*. Acute intravenous toxicity of both compounds in mice, rats and rabbits presents the same train of symptoms: increased respiration, tonic convulsions and death due to cardiac and respiratory failure. Partial heart block with an A-V rate varying between 2:1 and 7:1 was observed in all species. The LD_{50}'s in mice, rats and rabbits were: Toluidine Blue 27.56, 28.93, and 13.44 mg/kg.; Neutral Red 141.62, 112.42 and 96.6 mg/kg. Intravenous injection of 0.1 of the LD_{50}/kg. twice weekly into groups of three female rabbits showed that these doses produced no change in erythrocyte, leukocyte or differential counts or the hemogolobin or coagulation time of the animals during the 90-day observation period. Histological examination of the brain, heart, liver, pancreas, gall bladder, urinary bladder, spleen, lungs, adrenals and kidneys revealed that Toluidine Blue produced no evidence of histological damage. However, Neutral Red gave the following pathology in one of the chronic animals: marked, active lymphoid hyperplasia in the ileum; the sinusoids in the perifollicular area of the spleen were almost obliterated by intercellular deposits of hyaline material, but there were no such deposits in the follicle centers and the vessel walls; similar deposits were found in most of the glomerular tufts between the capillaries and although there were hyaline casts in many tubules, there was no tubular atrophy. (Work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the Univ. of California, Los Angeles.)

Inhibition of the renal tubular transport of phenolsulfonphthalein in the dog by dehydroacetic acid and its reversal by acetate. FERNLEY STONEMAN,* F. E. SHIDEMAN AND ROBERT C. RATHBUN.* *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Dehydroacetic acid (DHA), 2,4-dinitrophenol (DNP) and Carinamide are capable of inhibiting the renal tubular transport of both *p*-aminohippurate (PAH) and phenolsulfonphthalein (PSP). The effect of intravenous DHA (100 mg/kg.), DNP (10 mg/kg.), and Carinamide (25 mg/kg. plus 30 mg/kg/hr.) on the Tm of PAH in the dog is antagonized by the intravenous infusion of 100 μM/kg/min. of sodium acetate. Suppression of PSP secretion by the above inhibitors is not affected by acetate administered at the above rate (SHIDEMAN, F. E. AND R. M. RENE. *Am. Soc. for Pharmacol. and Exper. Therap., Fall Meeting, Abst.*, 1950). Operating on the hypothesis that both compounds employ the same secretory mechanism but that this mechanism has less affinity for PSP

than for PAH, experiments were performed to determine whether the effect of smaller doses of the above inhibitors could be antagonized by higher rates of infusion of acetate, i.e. by increasing the ratio of acetate to inhibitor. By decreasing the dose of DHA to 12.5 mg/kg. it was found that marked suppression of PSP secretion occurred and persisted for more than 2 hours. When acetate was administered during the DHA inhibition at a rate three or four times that used previously, PSP clearance could be returned to near normal levels. These findings appear to support the above hypothesis. Similar experiments with DNP and Carinamide are in progress.

Pharmacological and toxicological observations on antazoline (Antistine) hydrochloride. TULLIOUS C. STOUDEMAYER* AND ROBERT A. WOODBURY. *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

This histamine antagonist resembles others quite closely in its pharmacological effects. A progressive decrease of the amplitude of auricular and ventricular contractions, a decrease in the heart rate, and a prolongation of conduction time occurred as the dose was increased. Cardiac effects were observed with 1 mg. doses in frogs and turtles and with concentrations above 0.015 mg/ml. on cat's papillary muscles. Diphenhydramine (Benadryl) in similar quantities produced similar effects. Epinephrine, ephedrine and digitoxin did not protect against the cardiac weakness. In dogs antazoline produced mild or moderate toxic effects of rhinorrhea, excessive salivation, hyperirritability, muscle tremors, loose stools and urination. Accentuation of the above effects, ataxia, convulsions and cyanosis and death occurred with larger doses. The LD_{50} is 30 mg/kg. intravenously. The convulsive $dose_{50}$ in dogs is 45 mg/kg. orally and 25 mg/kg. intravenously. The integrity of the cerebrum is essential for the development of convulsions. Diphenylhydantoin calcium gluconate and glucose administrations provided no significant protection, though the barbiturates effectively prevented or eliminated the convulsions. Oral doses of 7.5 and 15 mg. administered two times daily over a 3-month period produced no significant effects on the blood picture, weight or general health of the dogs. Cumulative mild toxic effects sometimes were observed on the 4th–6th day of each 6-day week. At autopsy no significant effects were observed either grossly or microscopically. (Partially supported by a grant from Ciba Pharmaceutical Products, Inc.).

Effect of oxygen inhalation at increased ambient pressures upon tissue carbon dioxide tension. M. W. STROUD, 3rd,* C. J. LAMBERTSEN, R. H. KOUGH,* R. A. GOULD* AND J. H. EWING.* *Depts. of Pharmacology, Psychiatry and Medicine, Univ. of Pennsylvania, Philadelphia.*

Campbell (*J. Physiol.* 68: vii, 1929) and Taylor (*J. Physiol.* 109: 272, 1949) have described early sustained rises (30–200 mm. Hg) of pCO_2 in subcutaneous gas depots of small animals breathing oxygen at 2–4.8 atmospheres. We have performed similar experiments upon small animals and psychotic patients but have not found marked immediate rises of subcutaneous (tissue) pCO_2. The rises observed were slow in onset and accompanied by pulmonary damage and respiratory failure in the small animals. No appreciable increase of 'tissue' pCO_2 occurred in humans prior to the onset of oxygen convulsions. The rapid, marked rise of pCO_2 found by Campbell and Taylor is probably not due to loss of the dual function of hemoglobin but appears to be the result of other factors such as: *1*) pulmonary damage by oxygen and by decompression; *2*) respiratory depression; and *3*) diffusion of CO_2 into the depot during decompression of the animal for gas sampling. In our experiments gas sampling was performed at the pressure of exposure to oxygen.

Utilization of glucose in vitro by human brain slices. V. C. SUTHERLAND AND T. N. BURBRIDGE (introduced by H. W. ELLIOTT). *Div. of Pharmacology and Exptl. Therapeutics, Univ. of California School of Medicine, San Francisco.*

Samples of cerebral cortex or tumor were obtained from 9 psychiatric patients undergoing prefrontal lobotomy and from 6 patients undergoing removal of various types of brain tumors. The conventional Warburg manometric technique was used, so oxygen uptake could be determined as well as glucose utilization. Several substrates in Ringer-PO_4 were used: 0.2% glucose, 0.2% glucose plus 0.4% casamino acids (Difco vitamin free), and 0.2% glucose plus 0.26% ethanol. After 2 or 3 hours at 37° C. a tissue free aliquot of substrate was removed from the Warburg flask, and glucose was determined immediately on a protein free filtrate of the substrate. The glucose utilization of the histologically normal tissue from psychiatric patients was 0.0065 ± 0.0007 mg/mg. tissue/2 hours and 0.01124 ± 0.0044 mg/mg. tissue/3 hours in glucose Ringer-PO_4. With glucose-ethanol Ringer-PO_4 there was a significant increase in glucose utilization without change in total oxygen uptake. The utilization of glucose in glucose Ringer-PO_4 for neoplastic infiltrated or neoplastic tissues was not significantly different from the histologically normal tissue, although the total oxygen uptake was lower. With glucose-casamino acid Ringer-PO_4, the amount of glucose used was not significantly different than with glucose alone for the histologically normal and infiltrated tissues but was significantly lower for the neoplastic. The

oxygen uptake for other than the normal tissue was lower and significantly so for the neoplasms.

Effect of nephrectomy and liver injury on anticonvulsant activity of diphenylhydantoin. EWART A. SWINYARD, LAWRENCE C. WEAVER* AND LOUIS S. GOODMAN. *Depts. of Pharmacy and Pharmacology, Univ. of Utah, Salt Lake City.*

Diphenylhydantoin was tested in normal, nephrectomized and carbon tetrachloride-treated rats for its ability to prevent the tonic extensor component of maximal electroshock seizures. The drug was administered intraperitoneally 15 hours after nephrectomy or 48 hours after a single subcutaneous dose (1 gm/kg.) of carbon tetrachloride, and its anticonvulsant action determined at 2, 5 and 24 hours. Carbon tetrachloride-induced liver injury markedly increased both the potency and the duration of action of the drug. The most marked increases in potency were observed at the longer time intervals. In contrast, nephrectomy did not significantly alter the anticonvulsant potency of diphenylhydantoin. These data would appear to support the concept that the liver is a principle site for the degradation of diphenylhydantoin to substances devoid of anticonvulsant action, and that the maximal electroshock seizure technic provides a useful tool in the study of the metabolic fate of this compound. Analogous studies of related hydantoins and antiepileptic drugs from other chemical series are being conducted.

Site of cardiovascular action of hypotensive veratrum derivatives. EDWARD D. SWISS,* HOPE SIMON* AND J. W. STUTZMAN. *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Cross circulation experiments were performed to elucidate the hypotensive mechanism of protoveratrine and Veriloid. Fourteen experiments were performed in which Veriloid was confined successively to the head or the body circulation of the pentobarbitalized dog. Five similar experiments were performed with protoveratrine. Bilateral carotid cannulation of the recipient and donor was utilized to maintain the recipient's head. Isolation of the head from the body circulation was tested by isolation of dye in the body and by production of a fall in blood pressure in the recipient's body after the injection of epinephrine to the head. Cerebral venous return from the recipient was collected and re-infused into the donor. All injections to the recipient's head were made into the tubing connecting the carotids of the two dogs. Injections to the recipient's body were made into the femoral vein. Injection of 5 μg/kg. of Veriloid or 1 μg/kg. of protoveratrine into the head circulation caused a fall of blood pressure in the recipient's body without change of heart rate. This result was uninfluenced by vagotomy or atropinization. Injection of a similar dose into the body circulation caused a slowing of the heart and a fall of blood pressure before atropinization or vagotomy and not after it. It may be concluded that the site of the hypotensive action of these veratrum derivatives is central.

A comparative statistical study of the pressor effects of epinephrine and arterenol. VIRGINIA L. SYDOW,* RAYMOND P. AHLQUIST, JAMES P. TAYLOR* AND J. BAYARD BRITTON.* *Dept. of Pharmacology, Med. College of Georgia, Augusta.*

Though it has been shown repeatedly that l-arterenol is a more active pressor agent than l-epinephrine it is also known that on occasion l-epinephrine is the more active. Observations were made on a series of dogs to study the occurrence of this phenomenon. All dogs were prepared in a standard manner with morphine, pentobarbital, heparin and atropine. Blood pressure was recorded with a mercury manometer from the femoral artery. The drugs, l-epinephrine bitartrate and l-arterenol bitartrate, were injected into the femoral or jugular vein in a dosage of 0.01 cc/kg. of M/1000 solution. An initial 39 dogs (18 male, 21 female) have been studied with the following results (all pressures in mm. Hg): Epinephrine; mean control pressure 105 (range 75–133), mean pressor rise (MPR) 70 (range 24–132), S.D. ± 24. Arterenol; mean control pressure 106 (range 78–132), MPR 81 (range 24–118), S.D. ± 22. In 10 (25%) of the animals epinephrine produced a greater pressor rise; epinephrine MPR 92 (range 70–132); arterenol MPR 85 (range 65–115); mean difference 6 (S.D. ± 9). In 27 (70%) arterenol produced the greater pressor rise; arterenol MPR 81 (range 44–118; epinephrine MPR 63 (range 30–110); mean difference 18 (S.D. ± 12). In 2 (5%) dogs the drugs produced equal pressor rises. Additional dogs will be included in this report as well as a comparison of the pressor effects of continuous epinephrine and arterenol infusions. (This work was supported by a grant from the American Heart Association, Inc.)

Effect of suckling on adrenal ascorbic acid level of mice. IRVING I. A. TABACHNICK AND J. J. TRENTIN (introduced by D. D. BONNYCASTLE). *Depts. of Pharmacology and Anatomy, Yale Univ. School of Medicine, New Haven, Conn.*

Lactation in the rat and mouse is associated with a significant reduction of circulating leukocytes, which may be attributable to removal via the milk. In the mouse the leukocyte count doubled in 24 hours after removal of the suckling young, and fell to its former level within 3 hours of returning the young (GARDNER AND DOUGHERTY. *Anat. Record* 88: 432, 1944). The changes involved both lymphocytes and leukocytes. In view of the evidence for discharge of other pituitary factors

(pituitrin and prolactin) as a result of suckling, the possiblity of an adrenal involvement in the lymphopenia of lactation was investigated. Between the 5th and 12th day of lactation the young were removed from F_1 hybrid mice (C_3 H X A). After 24 hours the young were returned to half the mothers. One hour later all of the mothers were killed and adrenal asdorbic acid determined (Munson modification of Sayers' method). The mean adrenal ascorbic acid level for 39 suckled mice was 211 mg. %, while for 39 non-suckled mice it was 241 mg. %. Analysis of variance indicated a significant difference between days of assay as well as between treatments ($P < .001$ for each). The mean value for 6 mon-lactating mice was 284 mg. %. The mean value for lactating mice injected intraperitoneally with 3 and 6 mg. of histamine was 218 mg. % (6 mice) and 114 mg. % (14 mice) respectively. (Aided by a grant from The Jane Coffin Childs Memorial Fund.)

In vitro metabolism of histamine. HERBERT TABOR AND HUGO BAUER.* *Natl. Insts. of Health, Bethesda, Md.*

We have previously reported (*J. Biol. Chem.* January 1951) the preparation of a highly purified diamine oxidase (histaminase) from an acetone powder of hog kidney. Using histamine as the substrate, oxygen consumption measurements, aldehyde tests, and the diazo reaction indicated the probable formation of imidazole acetaldehyde. When either the DPN-linked aldehyde oxidase described by Racker (*J. Biol. Chem.* 177: 883, 1949) or xanthine oxidase was added to this reaction mixture, further oxidation occurred, indicating the probable formation of imidazole acetic acid. The product obtained from the coupled diamine oxidase-xanthine oxidase system was compared with synthetic imidazole acetic acid, which was prepared by hydrochloric acid hydrolysis of 4(5) cyanomethylimidazole. Chromatographic studies on Dowex 1-acetate, as well as comparison of the respective picrates, indicated the identity of the preparations. These findings indicate that the primary diamine oxidase reaction is an oxidative deamination of the side chain and does not involve the imidazole nucleus. Further studies have been carried out on the acetylation of histamine by pigeon liver (MILLICAN, R. C., S. M. ROSENTHAL AND H. TABOR. *J. Pharm. & Exper. Therap.* 97: 4 1949). An enzyme has been purified from pigeon liver acetone powder, which catalyzes the acetylation of histamine by synthetic acetylphosphate in the presence of transacetylase (STADTMAN, E. R. *Federation Proc.* 9: 233, 1950), coenzyme A and cysteine.

Separation of the positive inotropic actions of ouabain and fluorobutyrate. MICHAEL J. TAKOS* AND MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Quabain fails to produce a positive inotropic response in cat ($0.75 - 1.3 \times 10^{-7}$ M) and monkey (1.5×10^{-7} M) papillary muscle after failure has been produced by .0001–.0002 M thiopental (as the acid). No such phenomena are noted when sodium pentobarbital is used to produce failure. This ouabain-blocking action of thiopental does not extend to the positive inotropic effects of sodium fluorobutyrate (.001–.005 M) and protoveratrine (0.2–0.5/cc). Hence, there must be some difference in the site of action of ouabain as compared to fluorobutyrate and protoveratrine in the production of positive inotropic responses in the failed papillary. The ouabain-blocking action of thiopental is presumably not dependent on the presence of a thiol radical since sodium pentobarbital failure of the muscles in the presence of l-thiosorbitol, cysteine, and thiourea (.0005–.002 M) does not prevent the positive inotropic response to ouabain. Concentrations of sodium carbonate, a contaminant of thiopental, four times greater than those found in commercial samples failed to prevent the positive inotropic action of ouabain.

Distribution of Tridione (3,5,5-trimethyloxazolidine-2,4-dione) in the tissues of the mouse. J. D. TAYLOR AND E. L. BERTCHER (introduced by R. K. RICHARDS). *Dept. of Pharmacology, Abbott Labs., N. Chicago, Ill.*

A new micromethod was developed for the estimation of Tridione in tissues. The method was based on the breakdown of the oxazolidine-dione ring by NaOH. Upon acidification of the breakdown products, the CO_2 evolved was measured in the Warburg. It was possible to determine Tridione over a range of 0.25 – 1.25 mg. in 2 gm. of tissues. The persistence of Tridione in the organs of the mouse at various times after intraperitoneal injection decreased in the following order: liver = muscle > kidney = blood > brain. In mice given 1 gm/kg., the Tridione concentration in the brain fell at a constant rate of approximately 0.25 mg/gm/hr. It was interesting that although the concentration of Tridione in mouse brain had decreased below the limits of the method in about 4 hours, protection against Metrazol convulsions was present for periods longer than 16 hours. In the brain of the rabbit it was found that the concentration of Tridione was not significantly different in the gray matter, white matter, and the cerebellum.

Effect of convulsant and anticonvulsant agents on the activity of succinic dehydrogenase. CLARA TORDA AND HAROLD G. WOLFF. *New York Hosp., Cornell Med. Center (Depts. of Medicine (Neurol-*

ogy) and Psychiatry), and Kingsbridge Hosp. (V.A.), New York City.

The effects of convulsant and anticonvulsant agents on the activity of succinic dehydrogenase have been investigated. The activity of the enzyme increased in the presence of the convulsant agents used (acetylcholine, caffeine, camphor, dichlorodiphenyltrichloroethane, mecholyl, methylsalicylate, pentamethylene tetrazol, picrotoxin, scilliroside, and strychnine) in concentrations of 1×10^{-3} M. and less. The activity of succinic dehydrogenase was not modified significantly by the anticonvulsant agents used (hydantoin, methyl-phenyl-ethyl hydantoin, phenylhydantoinate sodium, phenobarbital, and Tridione) in concentrations of 1×10^{-3} M. and less. It is known that changes in the electrical activity of the brain characteristic for seizures precede the increase of cerebral oxygen consumption (DAVIES, P. W. AND A. REMOND. *Research Publ., A. Nerv. & Ment. Dis.* 26: 205, 1947). The above presented results together with the results obtained with other enzymes involved in oxidative processes (TORDA, C. AND H. G. WOLFF. *Proc. Soc. Exper. Biol. & Med.* 74: 744, 1950) indicate, therefore, that convulsant agents activate at least two processes: *1*) a non-oxidative mechanism that initiates the seizures; and *2*) the oxidative recovery processes.

Delayed fall in blood pressure following administration of vasopressin to pregnant dogs. JOSEPH H. TRAPOLD* AND ROBERT A. WOODBURY. *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

Reports of vascular collapse and fatalities following the administration of posterior pituitary solution to pregnant patients and vasopressin (purchased as Pitressin) in non-pregnant patients have appeared sporadically in the literature. Similar phenomena are described here in dogs in early pregnancy. The blood pressure response of 10 barbitalized, pregnant dogs to the intravenous administration of vasopressin was recorded from the carotid artery. Doses of 0.01 unit, 0.1 unit, and 0.32 unit/kg. of vasopressin were administered to each animal with an hour interim between each dose. Five of these 10 animals were studied when gestation had existed less than 4 weeks. These 5 animals were fairly refractory to the pressor action of the drug and 4 of them manifested a sustained fall in blood pressure which occurred not less than 15–45 minutes after the initial injection of vasopressin (0.01 unit/kg.). The fall in blood pressure in this group ranged from 16–130 mm. of Hg. When vasopressin was administered following Piperoxane Hydrochloride (933F) 5 mg/kg., to two of these animals an insidious fall in blood pressure occurred which terminated in death of both animals. The remaining 5 animals of this series were in the last half of gestation. They were only slightly, if at all, refractory to the pressor action of vasopressin and did not exhibit the sustained delayed fall in blood pressure that occurred in the animals in earlier pregnancy. Forty non-pregnant female dogs, similarly treated as above, did not exhibit the above type of blood pressure fall. Possible mechanisms to account for this fall will be discussed.

Effect of procaine on the squid axon (Loligo pealii). A. P. TRUANT (introduced by BYRON B. CLARK). *Dept. of Pharmacology, Tufts College Med. School, Boston, Mass.*

A simple perfusion apparatus for holding the squid giant axon and the electrodes was used to study the sequence of events that occur during conduction block. Three cells were made possible by means of two petroleum jelly seals, thus allowing the independent perfusion of three axon segments (I, II, III). The magnitudes of the action potential, threshold of excitability and conduction velocity of each segment were measured under conditions of a constant rate of perfusion. The giant fiber was stimulated by means of single shocks. Normally the middle axon segment was perfused with procaine solutions (0.0015–0.02 M). The effect of procaine was first manifested by a progressive increase in the threshold at II. After a short interval (30–60 seconds) the spike amplitude (II), initiated by a stimulus at I, declined progressively. Eventually the threshold (II) reached a maximum and the action potential (II) disappeared. The threshold and action potential response at I and III remained unaffected, with the exception of the conduction velocity at III which decreased 30–90 seconds before complete block at II. Threshold-time curves were of two types *1*) linear, *2*) exponential. The curves for the recovery phase of the threshold were consistantly of the latter type. The recovery sequence was also studied. Minimal effective concentrations of procaine were 0.0015–0.002 M. Similar effects have been seen in the frog sciatic nerve, for which the rate of release of procaine was found to follow changes in the threshold of the A fibers.

Measurement of analgesia in rats. PAUL E. TULLAR (introduced by RUSSELL A. HUGGINS). *Dept. of Pharmacology, Univ. of Texas, Dental Branch, Houston.*

Responses to a controlled spot of light focused on the tail tips of barbitalized rats offers a quantitative method of measuring hypo- and hyperalgesia. Standard (morphine) and unknown drugs may be compared in each animal of the series thereby minimizing the interference due to variations in individual sensitivities. The intensity of the projected beam is adjusted until it produces a sufficiently uncomfortable sensation that will

cause the barbitalized animal to flick its tail upon 10–15 seconds exposure. Further depression such as follows the administration of analgesic drugs is then measured by the increase in the exposure time which takes place before the tail is flicked: and increases in sensitivity may be measured by shorter exposure times. The method combines some of the features of the Hardy-Wolff-Goodell and D'Amour-Smith techniques with those introduced by the author: and is similar to the procedure used by the author in a study of local anesthetics on humans. Barbitalized rats handle easily, their responses to this type of painful stimulation is unmistakable, various types of controls may be readily included, and the procedure can be repeated indefinitely without trauma or significant change in sensitivity. Studies made with morphine and N-allyl-normorphine show the latter produces a morphine-like analgesia in doses approximately three times the effective mg/kg. morphine dose but is of much shorter duration. Morphine-like depression of respiration has not been observed at the doses used in this study.

Dog and rat as test animals for diuretic testing. C. Gordon Van Arman,* W. E. Hambourger and D. M. Green. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

Female dogs, following bladder drainage, were kept for 5 hours in metabolism cages without food except 60 gm. of Pard. Drugs, when given, were incorporated in the food. Water was removed during the experiment. Control and treatment experiments were made on successive days. Drug effects were expressed as treated minus control value, divided by body weight. Using 4 potent drugs, the standard error of this measurement in groups of 3 dogs was 1.35 cc/kg. for urine volume and 0.35 mEq/kg. for sodium excretion at effects up to 9 cc/kg. and 1.7 mEq/kg. respectively. In the rat method, groups of 8 were fed 25 cc. of saline/kg. with the drug. Urine was collected at 5 and 24 hours. Three groups were controls, 4 treated. Using three potent drugs, standard errors of the drug effects (expressed as in the dog method) at 5 hours were 1.93 cc/kg. at effects up to 13 cc/kg. and 0.22 mEq/kg. at effects up to 3.0 mEq/kg. At 24 hours, standard errors were 2.34 cc/kg. and 0.30 mEq/kg. at effects up to 12 cc/kg. and 2.6 mEq/kg.

Anti-curare effect of 3-hydroxypenyl dimethylethylammonium bromide (Ro 2-3198) in the rat. E. F. Van Maanen (introduced by G. H. Acheson). *Dept. of Pharmacology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.*

Curarization of the sciatic nerve muscle preparation was produced by intravenous injection of *d*-tubocurarine. Varying amounts of Ro 2-3198 (0.1, 0.3, 1.0, and 3.0 mg/kg. rat) given intravenously 30 seconds prior to the *d*-tubocurarine shift the dose-response curve of the latter only along the dose scale, so that the slopes of the curves stay within the fiducial limits of the control. The PD_{50} of *d*-tubocurarine, which was 50 μg/kg. in the control, increased to 91 μg/kg. after the administration of Ro 2-3198. The 19/20 confidence limits were respectively (37–67) and (70–120) μg/kg. rat. Since the anti-curare effect of Ro 2-3198 is constant over a 30-fold dose range, the effectiveness must be limited. Higher doses of Ro 2-3198 depress muscular contraction; its PD_{50} is approximately 15.0 mg/kg. rat and is not changed by the administration of 25 μg/kg. *d*-tubocurarine 90 seconds before the Ro 2-3198. The anti-curare effect of a large dose of Ro 2-3198 was tested by the determination of the PD_{50} of *d*-tubocurarine 0.5, 1.5, 5, 15 and 50 minutes after the injection of 10 mg/kg. Ro 2-3198. The results were respectively 15, 46, 70, 88 and 75 μg/kg. rat. Thus, after this large dose of Ro 2-3198, the sensitivity to *d*-tubocurarine is increased during the first 2 minutes; this is followed by an anti-curare effect which lasts for more than 50 minutes.

Quantitation of halogenated thiouracil in blood. Gilmour L. Wagle* and Albert J. Plummer. *Research Dept., Div. of Macrobiology, Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Since 5-iodothiouracil is precipitated in varying degrees by the usual protein precipitants, it cannot be determined directly on protein free filtrates. Iodothiouracil and thiouracil as well, when added to dog plasma, can be extracted completely by butyl acetate after first rendering the plasma acid with hydrochloric acid. The thiouracils are then removed from the butyl acetate by extraction with half normal sodium hydroxide. The extract is buffered at pH 7.0, is applied to Whatman No. 1 filter paper and developed by paper chromatography in a butanol water system according to the technique of Shabica and Solook (*Federation Proc.* 9:314, 1950). An examination of the chromatogram in ultraviolet light or after spraying with Grote's reagent not only locates but gives a good index of the relative amounts of iodothiouracil and thiouracil present and thus serves as a check on the quantitative results obtained after elution and subsequent spectrophotometric determinations. An additional check is afforded by the determination of total thiouracil directly on an aliquot of the buffered plasma extract. Iodothiouracil is precipitated with the protein bound iodine fraction using the Somogyi zinc hydroxide precipitation method for plasma proteins. It can be completely recovered by dissolving the precipitate in hydrochloric acid and extracting with butyl acetate as in the treatment of serum. Plasma

levels will be reported after the administration of iodothiouracil to the dog and man.

Bezold effect produced by several agents. HARRY A. WALKER, SUSANNE WILSON* AND ARTHUR P. RICHARDSON. *Dept. of Pharmacology, Emory Univ. School of Medicine, Emory University, Ga.*

The Bezold effect as first described with the veratrum alkaloids is a reflex phenomenon consisting of respiratory depression or apnea associated with bradycardia and hypotension, all abolished by vagotomy. We have observed this same response in chloralosed dogs with a number of agents listed below with their effective intravenous doses in mg/kg.: 3-hydroxy-2-phenyl cinchoninic acid (HPC), 5–10; phenindamine (Thephorin), 1–2; 2-methyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1-pyridindene (Nu 1525), 0.25–0.5; 1-diethylamino-3-o-tolyloxy-2-propanol (MC 2311), 2–4; and germitrine (veratrum alkaloid), 0.001–.002. Tachyphylaxis, well-known for the veratrum alkaloids, was quite marked with HPC in that dogs were immediately and almost completely refractory to a second dose and remained so for at least 8 hours. However, dogs refractory to HPC responded normally to the other agents mentioned above. Tachyphylaxis was either absent or of low magnitude with Thephorin, Nu 1525, and MC 2311. Special receptors sensitive to veratrum alkaloids have previously been located in the lungs, heart and carotid body area. It appears from data presented that the receptors sensitive to HPC and blocked by this agent for prolonged periods may be different from those sensitive to other agents. With the Heymans' technique of the perfusion of the isolated head the injection of effective doses of germitrine and HPC into the trunk of recipient dog B which is connected to the isolated head by the vagi alone produces the typical circulatory reflex effects. With this technique it is felt that the circulatory component of Bezold effect of germitrine may be separated from its other hypotensive effects.

Necrotizing action of various preparations for intramuscular injection. M. R. WARREN AND R. H. ALDEN.* *Div. of Pharmacology and Anatomy, Univ. of Tennessee Med. Units, Memphis.*

The intramuscular route of drug administration has been preferred to the subcutaneous route for prompter action and for the administration of insoluble or irritating preparations. The physical properties of the drug or the necessity for prolonged absorption often leave this route the only alternative. However, the frequent complaint of pain after injection, the prolonged soreness, and the reports of tissue damage at autopsy have led to the investigation of the necrotizing action of numerous preparations which are now available to the medical profession. A single dose of 0.05 cc. of each preparation was injected into the sacrospinal muscles of rats weighing 400 to 500 gm., using sterile technique. Seven days later the animals were sacrificed and examined for evidence of necrosis and tissue was preserved in Touin's fluid or 10% formalin. This study sought only to evaluate the degree and character of the tissue reaction which resulted from injection and did not attempt to relate it to the pH of the preparation, the vehicle, or the inherent properties of the injected materials. The preparations included representative analeptics, antibiotics, antihistamines, antispasmodics, antisyphilitics, blocking agents, hematinics, hormones, hypnotics, oxytocics, sympathomimetic amines, vitamins, and certain vehicles. The most extensive and severe necrosis was found among the analeptic, antibiotic, antihistaminic, antispasmodic, antisyphilitic, estrogenic, hematinic, hypnotic, and vitamin preparations. (Supported in part by a grant from the Committee on Research of the Council on Pharmacy and Chemistry of the American Med. Association.)

Effect of methanol, formaldehyde and formic acid on tissue respiration. D. T. WATTS. *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

There is some indirect evidence that methanol poisoning may be due to enzymatic inhibition by methanol or by its oxidation products, formaldehyde or formic acid. This concept has been tested by using the Warburg manometric method to determine the effect of a range of concentrations of methanol, formaldehyde and formic acid on *a*) the fermentation and oxidation of glucose by yeast, *b*) the respiration of rat liver slices in glucose, and *c*) the oxidation of succinate and ascorbate by fortified rat liver homogenates. It was found that at equal molar concentrations formaldehyde was a very potent inhibitor of the respiratory systems investigated whereas methanol and the formate ion had little or no effect on enzymatic activity. Typical of the results obtained were those on liver slices in which it was found that at a final concentration of 0.0156 molar, respiration was inhibited 65, 3, and 0% by formaldehyde, methanol, and formate respectively. This is considered to be *in vitro* evidence that formaldehyde is the toxic agent in methanol poisoning.

Stationary micro-respirometer for isolated mammalian muscle allowing simultaneous registration of mechanical activity. JAMES R. WEEKS* AND MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Sodium azide and 2,4-dinitrophenol have been reported to inhibit 'activity' but not 'resting' metabolism in various types of experiments. In order to extend such studies to mammalian skel-

etal muscle, a constant volume glass vessel was devised whereby oxygen consumption was measured manometrically as in the Warburg apparatus. This vessel was held stationary, and the liquid and gaseous phases kept in equilibrium by recirculating the oxygen (about 5 ml. total) through the liquid using a self-contained system of ball valves and hydraulically actuated pump. Strips of rat diaphragm were stimulated electrically and the contractile activity led out of the vessel on a human hair passing through a gas-tight packing consisting of a drop of mercury between two successive capillary constrictions. Dinitrophenol (10^{-6}M) caused about a one-third increase in resting respiration without changing the fiber length (direct recording). At 10^{-5}M, respiration was more than doubled and contracture produced. The onset of contracture was delayed about 30 minutes, but there was no significant difference in the respiratory rate before and after onset of contracture. Maximal isometric twitches (200/minute) for 30 minutes increased the mean Q_{O_2} of controls from 4.22–6.88. At the sub-contracture concentration of 10^{-6}M dinitrophenol, the muscles contracted normally and the increased oxygen uptake due to activity did not differ significantly from the control values. Similar results were obtained at sub-contracture concentrations of sodium azide (10^{-4}M), also indicating no inhibition of activity metabolism in mammalian skeletal muscle.

Comparison of the physiological disposition of Tromexan and Dicumarol in man. MURRAY WEINER,* GEORGE SIMSON,* J. J. BURNS,* SHEPARD SHAPIRO,* ELAINE L. KLEIN* AND BERNARD B. BRODIE. *New York Univ. Research and Med. Services, Goldwater Memorial Hosp., New York City, and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Tromexan (a carboxyethyl ester of Dicumarol) is absorbed more rapidly than Dicumarol, usually within 4–7 hours after oral administration. It is also metabolized more rapidly than Dicumarol so that usually within 8 hours after a single dose, the drug disappears from the plasma. As with Dicumarol, however, the rates of absorption and metabolic transformation vary considerably in different subjects. This explains the considerable individual variation in the height and duration of the plasma level of Tromexan in different individuals given the same mg/kg. dose. In addition, as previously noted with Dicumarol, the prothrombin response to the same plasma level of drug in different individuals varies widely. The delay in prothrombin response following the administration of the drug is less than after Dicumarol. However, the prothrombin response to a single dose is usually not evident until all the Tromexan has disappeared from the plasma. Progressively increasing prothrombin responses may result from repeated daily doses of the drug even though measurable plasma levels are present for only a fraction of the time. The implications of the above findings with reference to the relative merits of Tromexan and Dicumarol will be discussed.

Effect of a new derivative of a myocardial extract in cardiac conditions. ALLEN WEISS,* DAVID H. FELDMAN* AND FREDERICK STEIGMANN. *Cook County Hosp., Chicago, Ill.*

The effect of an orally administered new preparation derived from purified extract of beef hearts (Myocardone: Chemico Labs., Inc.) was studied in 58 patients with cardiovascular disease. Some of these presented evidence of cardiac decompensation, symptomatic hypertension, and/or angina pectoris, while others were compensated clinic patients. All acute cases had the usual clinical and laboratory workup, plus additional procedures if indicated. The preparation was employed in doses averaging 9 grains daily, and the patients were observed up to 6 months in some instances. Whenever feasible, all previous therapy was discontinued or temporarily withheld while the extract was administered and the need for nitroglycerine, digitalis, mercurials during this period was compared with that prior to it. In the compensated clinic patients the maintenance dose of digitalis or nitroglycerine was replaced with Myocardone. Definite subjective improvement resulted in two-thirds of the anginal cases; mild to moderate improvement in cases with symptomatic hypertensions; little clinical improvement in the acute decompensated cases; two of whom died. In some digitalized patients, who received Myocardone in place of digitalis, the compensation was maintained with no untoward effects. The subjective and objective clinical improvement was not always correlated with changes in blood pressure or E. K. G.

Action of ergot alkaloids on the middle meningeal artery of the dog. JOSEPH M. WHITE,* JAMES A. RILEY,* H. S. BURR* AND E. L. MCCAWLEY. *Dept. of Pharmacology and Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, Conn.*

In migraine the throbbing headache has been ascribed, by Wolf and others, to a painful dilatation of the dural vessels. An indirect, non-operative method for the study of the tone of the middle meningeal artery has been developed. The changing diameters of selected vessels, determined by measurements of photographs of the dura mater, have been compared with corresponding changes in the capillary bed of other ramifications of the external carotid artery in the cheek, recorded by

means of a photo-electric pulsimeter. A phototube in contact with the external surface of the cheek has been used to report variations in the amount of light transmitted by the tissues from a 'cold light' source against the internal surface. Increases and decreases occur as the blood content varies in each pulse cycle. Good correlations between these two measurements have been obtained. Nitroglycerine is said to initiate symptoms in 'migraine susceptible' patients. Under dial-urethane anesthesia, ergotamine, dihydroergotamine, dihydroergocristine, dihydroergocornine, and dihydroergokryptine have been tested by recording the effects of these agents on the vasodilator properties of nitroglycerine.

Metabolism of pressor amines. VIRGIL D. WIEBELHAUS AND KATHERINE M. WILHOYTE (introduced by KARL H. BEYER). *Dept. of Pharmacology, Med. Research Div., Sharp and Dohme, Inc., Glenolden, Pa.*

Phenol oxidase (potato) attacks epinephrine (*l* - (3,4 - dihydroxyphenyl) - 2 - methylamine - 1 - ethanol), arterenol (3,4-dihydroxyphenyl-2-amino 1-ethanol), adrenalone (3,4-dihydroxyphenyl-α-N - methylacetophenone), 'Synephrine'. (4 - hydroxyphenyl - 2 - methylamino - 1 - ethanol), isopropyl arterenol (3,4-dihydroxyphenyl-2-N-isopropylamino-1-ethanol) and the corresponding ketone (3,4 - dihydroxyphenyl - 2 - N - isopropylamino - 1 - ethanone), 'Epinine' (3,4 - dihydroxyphenyl - 2 - N - methylaminoethane) and the *p*-hydroxy homolog of 'Propadrine' (4-hydroxyphenyl-2-amino-1-propanol). The phenol oxidase does not oxidize 'Propadrine' (4-hydroxyphenyl-2-amino-1-propanol), 'Neo-Synephrine' (3-hydroxyphenyl-methylamino-1-ethanol), amphetamine (1-phenyl-2-amino-propane) or 'Aramine' (3-hydroxyphenyl-2-amino-1-propanol). At concentrations of 6×10^{-3} M the *d*-isomer of epinephrine is oxidized much less readily than the natural *l*-form. Racemic (*d*, *l*) epinephrine is oxidized as rapidly as the *l*-form. Apparently the para-phenol group is the essential functional group with respect to affinity for, and reactivity with, phenol oxidase. Our data indicate that metamonophenols do not form significant amounts of stable substrate-enzyme complexes. Substitution on the α-carbon of the side chain influences the rate of reaction by virtue of *a*) effect on the reactivity of the *p*-phenol group and *b*) the stereoisomeric position of the substituent (in the case of the alcohols). Preliminary studies on the *d* and *l* forms of arterenol show the same differential reactivity as the *d* and *l* forms of epinephrine. The *d* form of epinephrine is oxidized less readily by guinea pig liver oxidase than is the *l* form. Further data on the oxidation of pressor amines by amine oxidase will be presented.

Effects of large doses of N-allylnormorphine on man. ABRAHAM WIKLER. *Research Branch, USPHS Hospital, Lexington, Ky.*

In 12 post-addicts, subcutaneous injection of 30–75 mg. of N-allylnormorphine produced lethargy, mild drowsiness, vivid daydreams and dysphoria varying in intensity from vague anxiety to acute panic. The content of daydreams and degree of dysphoria varied in individuals and in the same subject on different days. All complained of inability to repress such daydreams, whose content appeared to be related to the degree of dysphoria. Miosis appeared in all subjects, pseudoptosis and sweating on forehead, palms and soles in most, while experiences of nausea, heaviness in the limbs, and hot and cold flashes were more variable. In 6 subjects the electroencephalogram remained essentially unchanged. In 2 others, in whom dysphoria was intense, alpha activity was sharply reduced. Intravenous injection of 30 mg. of morphine reduced only slightly the subjective changes produced by N-allylnormorphine. Intravenous injection of 0.1–0.2 gm. of sodium pentobarbital promptly and permanently abolished dysphoria and daydreaming but enhanced the drowsiness produced by N-allylnormorphine. Subcutaneous injection of 30 mg. of morphine 8 hours before, or 60 mg. of morphine 12 hours before, did not alter the effects of subsequent injection of 30–60 mg. of N-allylnormorphine. In 6 subjects, average changes during a 3-hour period after medication were as follows:

DRUG	TEMP. °C.	CARD. RATE PER MIN.	RESP. RATE PER MIN.	CIRC. EOSINOPHILES
(*a*) Morph. 30 mg.	−0.2	−2.7	−2.8	+36.9%
(*b*) N-allyl. 60 mg.	−0.5	−8.3	−1.7	−4.9%
(*a*) &, 1hr. later, (*b*)	−0.5	−3.3	−2.5	+16.5

Chronic toxicity studies of guanidineacetic acid and ethanolamine in rats. LUCILE WILLIAMS* AND HAROLD C. HODGE. *Univ. of Rochester, Rochester, N. Y.*

Transmethylation reactions are known to be important in choline metabolism. To test the hypothesis that transmethylation may be directly or indirectly the basic cause of the formation of tumors in choline-deficient rats, two types of methyl acceptors, guanidineacetic acid (GAA) and ethanolamine (ETA), were studied. The former (GAA) utilizes methyl groups without forming choline while the latter (ETA) forms choline. By supplementing the choline-deficient diet with two levels of each acceptor an attempt was made to show a relationship between growth,

extent of choline deficiency, and tumor formation. As temperature plays a role in the development of choline deficiency these studies were conducted at 21°C. and at 32°C. Weanling Wistar rats were distributed according to sex and litter-mates throughout 32 groups half of which were kept at 21°C., similar groups at 32°C. They were maintained for a period of two years on choline-deficient diets to which were added at levels of 1 and 6%, GAA, ETA, and a mixture of equal parts by weight of ETA and GAA (MIX). A choline-supplemented diet served as a control. All of the groups gained in weight. GAA depressed the growth more than ETA and equivalent to that of the MIX. A temperature of 32°C. depressed growth more than occurred at 21°C. Mortality decreased from GAA to MIX to ETA. Choline deficiency gave a higher mortality than a choline-supplemented diet. Temperature caused no consistent trend in mortality. Tumor incidence was not increased. Details of the pathology will be reported.

Parenchymal alkaline phosphatase reactions in livers of mice receiving sodium barbital, trional, or urethane. W. Lane Williams and Richard B Aronsohn (introduced by R. N. Bieter). *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Nuclei of normal mammalian liver cells are alkaline phosphatase (APh) positive and the cytoplasm negative. Clinically and experimentally, liver disease and injury of several types produce a strong positive APh reaction in the parenchymal cytoplasm, even with slight or no significant histological changes. The anesthetic doses of the three compounds were determined and adult mice received smaller amounts (as subcutaneous injections of aqueous solutions five times per week for 8 weeks) as follows: barbital 16 mg/100 gm. body weight; trional, 11 mg.; urethane, 33 mg. These doses produced hyperactivity and slight motor discoordination, but during the course of the experiments the mice seemed healthy and gained weight. At autopsy all viscera appeared normal, and a routine histological study revealed no significant alterations. APh was studied by a modified Gomori method. The reactions were normal (negative cytoplasm and positive nuclei) in the mice receiving barbital and trional. In urethane-treated mice there were positive cytoplasmic (and nuclear) APh reactions in 8 of 14 mice. This effect of urethane on the liver is of interest in relation to its nephrotoxic and pulmonary carcinogenic actions in mice, and also its 'inhibitory' action on hemopoietic tissues in several species, including man. These findings are preliminary within a study of the effects of anesthetics, analgesics and sedatives on the histochemistry of the liver.

Influence of metabolic inhibitors on the contractility of the papillary muscle. Martin M. Winbury,* W. E. Hambourger and D. M. Green. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

The influence of metabolic inhibitors on oxygen consumption, substrate utilization and contractility has been investigated with various heart preparations which may fail spontaneously. Results have not always been consistent with those of tissue slices or homogenates. The papillary muscle of the cat is well suited for such studies. It provides a thin strip of cardiac muscle which equilibrates rapidly with the bath medium and maintains contractility over long periods. For these studies, muscles were stimulated electrically in Krebs-Henseleit solution saturated with O_2-CO_2 mixture. Contraction force was recorded isometrically. Inhibitors were added directly to the bath. Cyanide produced a temporary increase in amplitude followed by depression. Cyanate and azide caused depression without initial augmentation. Recovery occurred on removal of the inhibitors but was not complete in the cyanate-depressed muscle. Spontaneous recovery was not observed. The 3,4-dinitrophenol depressed rapidly; considerable washing was required for recovery even after short exposure. Fluoride was followed by a gradual increase in amplitude which declined below the control value on washing. Fluoroacetate or iodoacetate produced progressive depression which was not influenced by drug removal. Fluoroacetate depression was preceded by a period of increased amplitude. Mercuric chloride depressed irritability and contractility without improvement on washing; BAL permitted complete recovery of irritability and partial recovery of contractility. Prophylactically, BAL prevented the depressant actions of mercury.

Pharmacology of the triiodoethylate of tri(β-diethylamino-ethoxy)-1,2,3 benzene (Flaxedil). Christian Wingard,* Walter F. Riker, Jr. and W. Clarke Wescoe. *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

One of the most prominent pharmacologic actions of Flaxedil is the production of neuromuscular blockade. In the intact anesthetized cat, the intravenous administration of 0.5 mg/kg. occasionally produces a fleeting potentiation of the muscular response to maximal motor nerve stimulation. Doses larger than this produce uniformly a depression of the response, the extent and duration of which are proportional to the dose injected; 2 mg/kg. suffice to paralyze completely. Recovery after doses of 1 mg/kg. is

achieved within 30–50 minutes. The 3-hydroxy phenyltrimethylammonium bromide (Ro 2-2561) antagonizes the curariform action of Flaxedil in a manner similar to that in which it antagonizes *d*-tubocurarine. Regardless of the rate of injection, the intravenous administration of Flaxedil in the cat, in doses as large as 10 mg/kg., does not modify the arterial blood pressure significantly. After doses as small as 0.1 mg/kg. a degree of vagal block is seen, and following doses of 0.5 mg/kg. the block is usually complete. The duration of vagolytic action exceeds that of the curariform action by more than an hour. The vagolytic action is characteristic and is localized at the effector level since the vagomimetic action of methacholine is prevented completely. Flaxedil, even in excessive dosage, is without effect on the responses of the gut, does not prevent salivation, and does not block autonomic ganglia. It is a moderately active inhibitor of cholinesterase (pKi50 about 1×10^{-5}M).

Effect of dinitrophenol on the heart and on its response to a cardiac glycoside. ALBERT WOLLENBERGER AND MAX L. KARSH (introduced by OTTO KRAYER). *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

Langendorff preparations of heart ventricles were continuously reperfused with 40 cc. of Locke-glucose solution at 39°C. The 2,4-dinitrophenol was administered until the amplitude of beat was reduced by 80%. The rate of beat remained undiminished. Coronary flow was somewhat increased. The final concentrations of dinitrophenol ranged from $0.9 - 1.5 \times 10^{-4}$M. These are concentrations which double oxygen consumption in guinea pig heart slices and suppress aerobic creatine phosphorylation in guinea pig heart homogenates. In the Langendorff ventricles they caused almost complete disappearance of phosphocreatine. The ATP content was not significantly changed. Administration to the poisoned preparations of 12 – 15 micrograms ouabain—an amount which normally had a marked positive inotropic action without producing toxic effects and which left the energy-rich phosphate content of the heart unchanged—produced no improvement of contractility and exerted a severe toxic action, causing arrhythmias, fibrillation, and contracture. This was associated with a 50% decrease in labile adenosine phosphate. The phosphocreatine level remained low. Coronary flow, though diminished, remained within the range observed in control preparations with high ATP and phosphocreatine content. These results support the hypothesis advanced by one of us (A. WOLLENBERGER. *Pharmacol. Rev.* 1: 311, 1949) that acceleration of the splitting of the terminal phosphate bond(s) of ATP in the myocardium may be an effect not only of toxic, but also of small, ordinarily therapeutic doses of cardiac glycosides, and that the advanced stage of cardiac glycoside poisoning is characterized biochemically by an increase in the rate of breakdown of energy-rich phosphate in the myocardium not compensated for by a corresponding increase in synthesis.

Comparative effectiveness of various sympathomimetic amines for the interruption of adrenergic blockade. ROBERT A. WOODBURY, MARSHALL R. WARREN, ARTHUR W. WALKER* AND ROBERT W. PREVOST, JR.* *Div. of Pharmacology, Univ. of Tennessee Med. Units, Memphis.*

It has previously been shown that certain sympathomimetic amines interrupt the blockade established by adrenergic blocking agents. This work has been extended to determine the relative potency of several amines on a molar basis. It was found that removal of the blockade established by Regitine or Dibenamine was more difficult than removal of the blockade by Priscoline or Benodaine. Listed in order of decreasing efficacy ephedrine, Paredrine, Vonedrine and Amphetamine removed the depressor effects and reestablished the pressor action of epinephrine. Tuamine did not modify the depressor effect and had little, if any, influence on the pressor action. The failure of ephedrine to potentiate the depressor action of epinephrine in these dogs demonstrates that the primary action of ephedrine is not the inhibition of aminoxidase. These data indicate that receptor mechanisms innervated by the sympathetic system can be altered either toward a state where excitation causes mainly dilation or toward a state where excitation produces mainly constriction. It is conceivable that at least three receptor mechanisms may be involved in these particular drug responses; i.e. adrenergic, cholinergic and histaminergic. The response may represent the sum of the component actions on each of these receptors. The amounts of these component portions vary from species to species and can be altered by drugs or physiological states. (Supported by a grant from the American Heart Association.)

Respiratory depressant action of some local anesthetics in anesthetized laboratory animals. L. A. WOODS, JANE HAGGART* AND J. COCHIN.* *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

The intravenous injection of 3 to 5 mg/kg. of cocaine hydrochloride into etherized dogs (plane iii anesthesia) produces an abrupt and irreversible cessation of respiration. Such a dose represents a 3- to 4-fold increase in toxicity as compared to cocaine administered to unanesthetized dogs. Simultaneous EKG, blood pressure and pneumographic recordings indicate that death results

primarily from respiratory failure. The supervening anoxia secondarily leads to cardiac irregularities and vascular collapse. If the dog is anesthetized with ether-oxygen, cardiac irregularities and vascular failure may not appear for as long as 8 or 9 minutes after injection of cocaine, even though there is a complete absence of respiratory effort. Bilateral vagotomy does not alter this reaction to cocaine. Intravenous injection of cocaine into the tail veins of etherized Webster strain Swiss mice (respirations 7–12/5 seconds) likewise demonstrates a 3- to 4-fold increase in toxicity of this local anesthetic. Again death is a result of respiratory failure. These observations are not peculiar to cocaine but also hold for procaine and lidocaine (Xylocaine). Mice deeply anesthetized with thiopental exhibit the same increase in the toxicity of cocaine although preliminary experiments indicate such is not true for mice anesthetized with pentobarbital. The depth of anesthesia is of considerable importance in demonstrating this increase in toxicity of local anesthetics inasmuch as dogs or mice under light plane i ether anesthesia are protected against doses toxic to the unanesthetized animal.

Direct application of tetanus toxin to the central nervous system. E. A. WRIGHT (introduced by OTTO KRAYER). *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

Tetanus toxin was injected directly into the brain and spinal cord of rabbits. The dosages of these injections necessary to cause death were compared with the intravenous dosages necessary to produce death in a similar time. Toxin injected intracerebrally was several thousand times more potent than when injected intravenously. The comparable potency for the region of the medulla oblongata was somewhat less than for the intracerebral injections. Toxin injected into the lumbar cord was several hundred times more potent than when injected intravenously.

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

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(An asterisk following an author's name indicates "by invitation")*

Use of wild house mice in studies on the mammary tumor agent. HOWARD B. ANDERVONT. *Natl. Cancer Inst., Bethesda, Md.*

The mammary tumor agent is found in inbred strains of mice which show a high incidence of mammary tumors and is, presumably, absent in strains which show a low incidence of these tumors. Since virtually all investigations to date were performed with inbred animals or their derivatives, it was decided to utilize wild mice to ascertain *1)* whether they were susceptible to the agent, and *2)* whether they harbor a mammary tumor agent. The wild mice used in these studies were derived from animals kindly supplied by Dr. H. A. Schneider of the Rockefeller Inst. for Med. Research. The agent from inbred strains increased the incidence of mammary tumors in wild mice. It was also found that the agent could be transferred through at least 4 generations of wild mice without producing tumors in them. The occurrence of mammary tumors in a family of inbred mice derived from a female who was suckled by a wild mouse, suggests that the wild mouse carried a mammary tumor agent of low activity.

Electron microscopy of ATP-induced contraction of skeletal myofibrils. CHARLES A. ASHLEY,* KEITH R. PORTER,* DELBERT E. PHILPOTT* AND GEORGE M. HASS. *Rush Dept. of Pathology, Presbyterian Hosp., Chicago; Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago; Rockefeller Inst. for Medical Research, New York City.*

Skeletal myofibrils isolated either by tryptic digestion at 0° C. or by a colloid mill and suspended in buffer solution (pH 7.0; μ 0.154) containing 20% glycerine and 0.0025 M ATP at −5° C. contracted slowly and progressively when the temperature was raised above 0° C. Formalin fixation halted this contraction. Myofibrils in progressive stages of contraction were studied with the electron microscope. Electron micrographs showed that fibrils isolated by the colloid mill were similar to those described by others. Fibrils isolated with trypsin differed by absent Z bands, absent or disorganized I bands and more evident filamentous structure. Changes induced by ATP were similar to those described by classical histology and earlier work with the electron microscope. Contraction was characterized by migration of dense material within the A band into the A-I junction or Z band, when present, a gradual increase in width of the fibril and a disorientation of fllaments from their parallel arrangement. The changes were similar in fibrils isolated by both methods. Neither the Z band nor an organized I band were necessary for contraction. Measurements of filaments adjacent to the myofibrils revealed an apparent increase in mean diameter during contraction.

Electron microscopic study of elastic tissue and collagen treated with formic acid. JOHN P. AYER,* GEORGE M. HASS AND E. C. PHILPOTT.* *Rush Dept. of Pathology, Presbyterian Hosp., and Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

During extraction of rings of canine aorta with 88% formic acid at 45° C. for 96 hours, all structural components except elastic tissue are dissolved. Upon neutralization, the elastic tissue exhibits perfect elasticity, great physical extensibility, good tensile strength and natural microscopic and staining properties. When immersed in formic acid, the networks of pure elastic tissue extend in direct proportion to the concentration of formic acid. The curves of chemical extensibility parallel the curves of physical extensibility, drawn from data obtained by application of increasing loads. Fibrils extended by formic acid become easily separable. Electron micrographs of the extended elastic fibrils disclose them as linear branching threads. They have a faint longitudinal striation but no consistent transverse periodicity or spiral arrangement is detectable. Upon neutralization, the fibrils retract into structures too dense for accurate electron microscopic study. Collagenous fibrils from canine tendon, fixed with formalin and treated briefly with 30–100% formic acid, are converted after neutralization and drying into spiral structures with intercoil dimensions proportional to the strength of formic acid used. Electron micrographs of the spirals show changes in the usual periodicity

of collagen with evidence of relations between loss of normal periodicity and acquisition of spiral structure. Similar fibrils, if unneutralized, show complete loss of periodicity.

Selective destructive effect of eastern equine encephalomyelitis virus on rat and human fibroblast cell lines. FREDERIK B. BANG AND GEORGE O. GEY. *Depts. of Medicine and Surgery, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

The virus of eastern equine encephalomyelitis has been grown on some eight lines of rat normal and malignant cells in the same medium of 10% beef extract and 50% human cord serum on glass without a plasma clot. Within this group of cells there is a wide spectrum of effect. Some lines are promptly killed, others support continued growth and others support little or no growth. Our first experiments compared a normal cell (14pf) with its tumor cell derivative (T333). The first normal cell was resistant in that infection was either not established at all or remained minimal for a few days with no cell destruction. The malignant cell succumbed promptly to a minimal inoculum of the virus and yielded a large amount of virus while the cells survived. Several other cell lines showed an intermediate type of susceptibility characteristic of these lines. Here virus and cells multiply continuously together. Some cells may be destroyed, virus in small amounts is continuously present in the supernatant and cell multiplication continues at a good rate. Electron microscope shows degrees of cell destruction. Cellular processes may be eaten away by virus with local disintegration of the progress. Other cells show closely packed rows of individual virus particles along the thin edge of the partially extended cell. Finally, half of a projecting part of the cell proper may be destroyed leaving the other half morphologically intact. Complete disintegration of the cell is accompanied by a scattering of disintegrated and vesicular components.

Cholesterol esters of lymph in experimental liver injury. JESSE L. BOLLMAN AND EUNICE V. FLOCK. *Div. of Exptl. Medicine, Mayo Fndn. Rochester, Minn.*

Intestinal lymph and liver lymph were drained through small polyvinyl catheters inserted in the main intestinal lymphatic or in the main liver lymphatic near their entrance into the cysterna chyle. From the fasting rat the lymph contained cholesterol and cholesterol esters in proportions similar to that of the plasma. When diets containing free cholesterol were fed the intestinal lymph cholesterol was increased with larger amounts of esterified cholesterol than of free cholesterol and there was little change in the cholesterol content of the liver lymph. From rats with experimental liver injury produced by repeated administration of carbon tetrachloride the lymph from the liver was usually found to contain relatively more free cholesterol than the plasma or intestinal lymph. After feeding free cholesterol to rats with liver injury the esterified cholesterol of the intestinal lymph is increased similarly to that observed in normal rats after cholesterol feeding. The liver lymph and to a lesser extent the plasma of rats with liver injury was found to contain more free cholesterol with little change in the cholesterol esters.

Transfusion transfer of experimental atherosclerosis. JOSEPH H. BRAGDON. *Natl. Insts. of Health, Bethesda, Md.*

Following single intravenous injections of serum or plasma from cholesterol-fed rabbits into normal rabbits microscopic lesions identical with those of early rabbit atherosclerosis may be observed in the recipient. The arterial lesions are focal and show a distribution similar to that in the cholesterol-fed animal. They have been observed as early as 5 hours after transfusion and as late as 2 weeks. Plasma produces more extensive lesions than serum; and oxalated plasma, more than heparinized plasma. Fractionation studies now in progress, designed to determine the factor or factors in the donors' blood responsible for the lesions, will be reported.

Isolation and characterization of a hemolytic substance from commercial trypsin. JOHN J. BUCKLEY (introduced by G. DALLDORF) AND FRANK MALTANER. *Div. of Labs. and Research, New York State Dept. Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

It has long been known that preparations of powdered pancreas are hemolytic. This was presumed to be due to the lipases present in the pancreas. In 1936 Gronchi (*Sperimentale* 90: 223) was able to isolate the lytic substance and claimed that it was a lecithinase A. We found that commercial trypsin T-360 (Eimer and Amend) had the ability to lyse sheep erythrocytes. If, however, the powdered preparation was thoroughly extracted with a mixture of benzol and alcohol, it lost this property. The Soxhlet-extracted preparation regained its activity if mixed with pure cephalin, whereas pure lecithin had no activating effect. The application of Gronchi's method to our preparation of commercial trypsin resulted in the isolation of a substance having the same qualities as were found in the original material. The substance could be activated by pure cephalin but not by pure lecithin. Furthermore, no lysobodies could be demonstrated. Crystalline trypsin and chymotrypsin showed no evidence of hemolytic activity either alone or in the presence of cephalin.

Pathological study of skin carcinogenesis in the albino rat. PERIHAN CAMBEL (introduced by JACOB FURTH). *Cancer Research Lab., Univ. of Florida, Gainesville.*

Previous studies showed that the rat is very susceptible to the action of various carcinogens when administered subcutaneously but it is one of the refractory species for skin carcinogenesis. 20-Methylcholanthrene (MC) is a carcinogen known to induce readily epidermal carcinogenesis in Swiss albino mice when administered according to a standard procedure. MC and 9,10-dimethyl-1,2-benzanthracene (DMBA) were administered to female and male Sprague-Dawley-Holtzman albino rats according to this procedure. No gross changes or skin tumors in the skin of either sex were observed in the group treated with MC. In the DMBA-treated group, hyperemia, accompanied by itching, thickening of the skin, epilation and subsequent hyperkeratotic crusts were observed previous to the development of warty excrescences. These latter showed progressive growth and invariably turned malignant after a long period of incubation. The incidence of warty excrescences and malignant tumors was slightly higher among the females. Histologically, the tumors were epidermoid acanthomas. A few showed lymph node metastasis, and one was accompanied by a subcutaneous sarcoma. Histochemical studies were made (alkaline phosphatase, glycogen, mucin reactions, nucleic acids). The results of this investigation lend support to the author's previous postulation that definite barriers exist in the albino rat which prevent the penetration of MC to the subcutaneous fat tissue and the basal cell layer of the epidermis from the sebaceous glands. The mechanism of the chemical induction of epidermal carcinogenesis in the rat, the role of the sebaceous glands, and the relative carcino-resistance of this species to cutaneous MC administration are discussed.

Electron microscope investigation of transverse and longitudinal sections of muscle. CECILY M. M. CANNAN (introduced by C. P. RHOADS). *Sloan-Kettering Inst., New York City.*

Earlier workers have studied striated muscle extensively by electron microscopy with the aid of various methods of fragmentation, disclosing the existence and ultrastructure of the myofilament and gross striations. Sections have also been examined but without sufficient resolution to demonstrate clearly the organization of filaments within the fibril or the nature of the sarcolemma. Smooth muscle had not been studied by either method, nor had the paramyosin fibril been demonstrated in intact muscle. These subjects were, therefore, open to certain speculations and were investigated in the following manner. Clam adductor muscle was prepared by methods known from diffraction studies to preserve submicroscopic structure optimally and thence fixed in formalin or osmic acid or preserved in glycerol. The specimens were embedded in paraffin and celloidin and then sectioned in an adapted Spencer microtome fitted with a hand-sharpened steel knife. Longitudinal sections clearly resolved myofilaments identical to those seen in fragmented material but disclosed no striations of the order of 400A. Paramyosin fibrils were noted distributed at random with respect to myofibrils. Transverse sections showed that the filaments are packed solidly within both the paramyosin and myofibrils. The sarcolemma has variable thickness, contains amorphous material and is continuous with myofibrillar membranes. The appearance of sections after different fixations and after alcohol extractions was correlated with diffraction studies and pertinent biochemical information. Similar treatment of smooth and striated muscle not containing paramyosin illustrated the general application of these morphological studies on clam muscle.

Cataract induction by slow neutrons and x-rays. K. W. CHRISTENBERRY* AND J. FURTH (with assistance of J. J. LANE). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

The causation of cataracts by large doses of x-rays and of fast neutrons by atomic bomb is known. Nothing is known of cataract induction by slow neutrons. Mice were placed in the thermal column of the atomic pile with a flux of slow neutrons of .9 x 10^9 n/cm^2/second free from fast neutrons, but with an estimated 4–6 r/minute contamination of gamma rays of high energy. It required 80 minutes exposure of mice in this flux to administer an LD_{50}. The equivalent LD_{50} of x-rays was 512 r administered at the rate of 6.4 r/minute. Groups of mice 8–10 weeks of age were exposed during 80, 20, 5, and 2.5 minutes respectively to x-rays or neutrons under the conditions named. The onset and progression of nuclear opacities were studied by the slit lamp microscope. The data indicate that neutrons induce cataracts sooner and in a somewhat higher percentage of mice than equivalent doses of x-rays. At 14 months after exposure almost all mice exposed to x-rays or neutrons during 20–80 minutes had some opacities of the lens, but only those exposed during 80 minutes had advanced lesions. At onset radiation cataracts arise in the posterior subcapsular region from which they extend into surrounding areas of the lens, while senile cataracts develop in the nucleus and cortex of the lens. The difference in cataractogenic effect between neutrons and x-rays was not evident as concerns: *a*) acute hemopoi-

etic changes including reticulocyte counts, *b*) weight changes, and *d*) leukemia induction.

An improved historadiographic apparatus and its application. J. J. CLEMMONS*, M. H. APRISON* AND D. M. ANGEVINE. *Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.*

X-rays cannot be refracted in any kind of optical system analogous to that in the light of electron microscopes. With direct contact between the sample and a suitable film, the exposure to extremely soft x-rays produces images of the histological section that can be subsequently enlarged. This procedure is called historadiography. Lamarque (*Radiology* 27: 563, 1936) introduced historadiography. A. Engstrom and B. Lindstrom (*Biochem. et Biophys. Acta* 4: 351, 1950), by the use of x-ray absorption laws, found it possible to calculate the absolute weight per unit surface of structure, as small as one micron in diameter. The original instruments required long exposure times and it was also necessary to break the vacuum between historadiographs. The instrument has been modified so that ten historadiographs can be obtained within one hour. The apparatus consists of a continuously evacuated demountable x-ray tube. Built into the tube is a chamber with a vacuum lock to permit a camera, containing the sample and film, to be introduced and removed without cooling the diffusion pump. With the use of Lippman film, a 30–40 second exposure is required when operating the x-ray tube at 3 kv. and 30 ma. This technique has been used to observe changes in the density of the cartilage matrix during the early stages of calcification in the developing tadpole and in young rats. This has been correlated with hematoxylin and eosin and the Von Kóssa stain for calcium. The versatility of the apparatus, and ease with which it can be used, suggest a wide application for it in pathology and histochemistry.

In vivo and in vitro effects of a lipid inhibitor of blood clotting. ROBERT W. COON, JOSEPH E. FLYNN AND PHILIP S. VASSAR (introduced by H. P. SMITH). *Dept. of Pathology, College of Physicians and Surgeons, Columbia Univ., New York City.*

The *in vivo* effect of lipid inhibitor (so-called 'antithromboplastin' described by Tocantins *et al.* and independently by Overman and Wright) was studied in dogs. The inhibitor was prepared from acetone dried beef brains using cold methyl alcohol and ether. The waxy residue from the ether extractions was prepared as a 2% emulsion in normal saline by means of a Waring Blender. Varying quantities of this emulsion were injected intravenously into dogs without apparent untoward reaction. Factor V assays, by the method of Flynn and Standley, showed prompt decrease, often as much as 75%; the prothrombin levels, as assayed by the 2-stage method with added factor V, also decreased as much as 70%, depending on quantity injected. Quick's prothrombin time test was less sensitive, with prolongation occurring only after larger doses. The decrease in factor V and prothrombin, and the length of recovery time could be directly correlated to the quantity of inhibitor injected. With larger quantities recovery was not complete in 24 hours. Similar results were obtained with rabbits. Although *in vitro* incubation of plasma with the inhibitor showed a decrease in prothrombin and factor V, the decrease was not as marked as *in vivo*, nor was the effect as prompt. No electrophoretic changes in dog plasma were noted after the injection. The lipid inhibitor may have an important role in anticoagulation therapy.

Toxic materials extracted from streptococcal skin lesions. WILLIAM J. CROMARTIE AND DENNIS W. WATSON (introduced by JEROME T. SYVERTON). *Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minn.*

Properties associated with sedimentable (12,000–14,000 rpm) and soluble fractions of extracts of streptococcal skin lesions have been studied and compared with properties of fractions obtained from extracts of unifected rabbit skin. The sedimentable fraction is similar to sediments prepared from other tissues by Thomas (*Bull. Johns Hopkins Hosp.* 81: 1–25, 1947). The soluble component was concentrated by dialyzing the supernatant fluid against distilled water and separating a euglobulin-like fraction. This fraction dissolved in 0.85% NaCl buffered at pH 7.4 with phosphate at a total ionic strength of 0.3 exhibits properties similar to 'necrosin' (Menkin, V. *Newer Concepts of Inflammation*. Springfield, Ill.: Thomas, 1950) and properties similar to the sedimentable fraction. Although small quantities of soluble toxin were obtained from uninfected skin, comparative determinations indicate that a larger yield resulted following the extraction of streptococcal lesions. Therefore, it is postulated, that the interaction of the parasite and host results in liberation of this soluble toxic material from the insoluble sedimentable toxic material. Injection of sublethal amounts of either the soluble or the sedimentable materials increases the tolerance of animals to a lethal dosage. Since this increased tolerance is associated with a prolongation of blood coagulation time and can also be induced by injection of heparin, it is suggested that the responsible factor is the liberation of heparin-like substances. The role that these toxic substances may play in the production of certain nonspecific reactions to injury will be discussed.

Influence of intestinal bacteria upon choline in normals and patients with hepatobiliary dis-

eases. J. DE LA HUERGA* AND HANS POPPER. *Hektoen Inst. for Med. Research of the Cook County Hosp., Chicago, Ill.*

The fate of administered choline in the human body was investigated because of its therapeutic effect as a lipotropic agent, and to investigate, if possible, hepatic function. Normals and patients with hepatobiliary diseases who were given from 2-8 grams of choline by mouth, excreted in the urine insignificant amounts of choline without relation to the hepatic condition (less than 0.3% of the amount ingested). Nevertheless, large amounts of a crystalline Reineckate were obtained from the urine after choline administration, mostly trimethylamine oxide and only small amounts of trimethylamine. The excretion of trimethylamines was markedly depressed if large amounts of cane sugar and even more so starch were given; glucose has been shown to suppress *in vitro* the trimethylamine formation from choline by intestinal bacterial. This excretion was also significantly diminished after previous treatment with Aureomycin and Sulfaphthalidine. Moreover after intravenous injection of choline, considerable amounts of choline, but not trimethylamines were excreted in the urine. These observations indicate that trimethylamines excreted (which have no lipotropic activity) were formed from choline by intestinal bacteria. Normals excreted more than 60% of the ingested choline as trimethylamines while patients with severe liver diseases excreted less. Patients with low urinary excretion of trimethylamines after choline ingestion, excreted fed trimethylamine rapidly and almost completely (in the oxide form). This indicates that reduced trimethylamine excretion in liver disease is an indication of an alteration of the intestinal flora in hepatic diseases.

Effect of altered lipid metabolism on experimental lesions of the coronary arteries. G. I. DESUTO-NAGY AND L. L. WATERS (introduced by HARRY S. N. GREENE). *Dept. of Pathology, Yale Univ. School of Medicine, New Haven, Conn.*

Local injury to the coronary arteries and a systemic disturbance of the body's own lipid metabolism were produced simultaneously but by separate means. The interaction of two such injuries was accomplished with the aid of allylamine and detergents. Either one of these agents, alone, produced only the usual response. Superimposition of the two injuries, however, led to the appearance of coronary lesions, with local accumulation of lipids in the intima and media of the vessel wall. The coronary lesions arose at the site of the primary local injury within a few days or weeks. The stepwise development of the experimental vascular disease will be illustrated, discussed and compared with similar lesions in man. These experiments will serve as models for the study of the pathogenesis of arteriosclerosis in man. They suggest that similar lesions may rapidly arise from a combination of the two factors: local vascular injury and a coincident disturbance of the lipid metabolism.

Expulsion factor for leukocytes in bovine plasma. ALBERT A. DIETZ,* BERNHARD STEINBERG AND RUTH A. MARTIN. *Toledo Hosp. Inst. of Med. Research, Toledo, Ohio.*

A previous report (STEINBERG AND MARTIN. *Am. J. Physiol.* 161: 14, 1950) described the presence of an 'Expulsion factor for leukocytes' in human plasma. The factor was heat labile and was precipitated by ammonium sulfate at a concentration of 62–68%. Injection of the substance into rabbits produced a peripheral leukocytosis and marrow hypoplasia. In an effort to find a ready source of this substance, the plasma of various animals was tested. This investigation concerns bovine plasma. Results produced by 17 samples of plasma or serum were analyzed. Eleven contained the expulsion factor. There was suggestive evidence that plasma contained factors acting on all the formed elements of the blood. Ammonium sulfate was not effective in separating them. Some rabbits appeared refractory to the action of the expulsion factor. The animals showed a neutralizing substance in their plasma for the expulsion factor.

Relationship between blood plasma proteins in health and in disease. E. E. ECKER AND J. P. FRACKELTON. *Western Reserve Univ., Cleveland, Ohio.*

A close relationship exists, in health, between the three main constituents of human plasma, namely; albumin, globulin and fibrinogen. This relationship has been expressed in a single constant 'K', as follows: $A^{0.62} \cdot G^{0.328} \cdot F^{0.052} = K$. 'K', under the above conditions, has *one* maximum value so that any change or combination of changes in A, G or F will result in a value which is less than the maximum which is 0.442+. This value was obtained from a study of 80 Tiselius diagrams from apparently healthy persons 20–45 years, and correlated with values found in 95 diagrams of younger, and 21 patterns of older age groups 70–95 years. 'K' varied 0.005 in the ages 0–45 years with an average of 0.441 for these years. In the age groups 20–45 years, the variation was only 0.002. It falls to 0.435 in the group 70–95 years. The 'K' value has been determined in essential and malignant hypertension, portal cirrhosis, nephrosis and nephritis, cardiac failure, tuberculosis, rheumatic fever, diabetes mellitus, hypo- and hyper-thyroidism and in tumors. Any change affecting one or more of the three factors was immediately reflected in a fall of the 'K' values.

Effect of flavonoids on transplanted tumors. W. H. EDDY, B. SOKOLOFF, J. WILLIAMS AND L. SCIORTINO (introduced by LEO LOEB). *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

The disclosure of two flavonoid compounds present in the adrenal gland (Sokoloff and Eddy) suggested an investigation on the effect of flavonoids on malignant growth. August Rat Carcinoma, 175-G served for these studies. Altogether 130 rats were tested, with an average weight of 154 grams. In the first series of experiments a flavonoid compound ($C_{36}H_{30}O_{11}$), water soluble, was injected subcutaneously, 10 mg/100 gm/weight for 19 days, 5 days prior to transplantation and for 2 weeks after. At the end of 3 weeks after transplantation the average size of tumor (for 20 rats) was 3.4 cm.^{3} against 6.4 cm.^{3} of control. This inhibition was a temporary nature. Twelve rats, all females, of average weight 151 gm., were grafted subcutaneously with two extra adrenal glands. The following day the tumor was transplanted. There was no significant difference in growth. At the end of 4 weeks the average size of tumor was 8.7 cm.^{3} against 9.1 cm.^{3} of control (10 rats). In the next series, 12 rats grafted with two extra adrenal glands were given injections of the flavonoid compound from the day of transplantation. There was a marked inhibition, of a temporary nature. At the end of 4 weeks the average size of tumor was 2.5 cm.^{3} as against 8.6 cm.^{3} of control. Discussion about the possible relationsh p of flavonoids and the adrenal gland.

Effect of the alarm reaction upon lymphocytes and plasma cells. WILLIAM E. EHRICH, JOSEPH SEIFTER AND GEORGE H. HUDYMA. *Graduate School Med., Univ. of Pennsylvania, Philadelphia Gen. Hosp., and Wyeth Inst. Appl. Biochem., Philadelphia, Pa..*

The lymphocytolytic effect of the alarm reaction is well established. Its action upon the thymus is used by us as a standard method to test toxicity. The present report deals with the effect of the alarm reaction upon various components of various lymphatic tissues. Young rats weighing 60 grams were given cortisone, formaline, co chicine, adrenaline, dibenamine and other materials. A study was made of the small lymphocytes of thymus, lymph nodes and spleen, the cells of the germinal centers of lymph nodes and spleen, and the plasma cells in the medullary cords of lymph nodes. The results of these experiments will be presented and the significance of lymphocytolysis will be discussed.

Effect of vitamin B_{12} and aureomycin upon the toxicity of chloroform to regenerating liver. BERNARD FISHER* AND HARRY M. VARS. *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

Adult male Wistar rats were fed a non-protein diet for 14 days, subjected to 70% partial hepatectomy, and offered an 18% fibrin diet. Group A received 10 mg. aureomycin per gram of food eaten. Group B was injected with 2 γ vitamine B_{12} on alternate days (total dose 8 γ), and group C received saline injections. On the 4th postoperative day all received a subcutaneous injection of chloroform (1.2 ml/kg.) in mineral oil (1 ml. volume), and were sacrificed 4 days later. All groups ate approximately the same amount of food. There was no significant difference between the groups in the amount of liver protein regeneration; or in the histological picture as observed by hematoxylin and eosin, or methyl-green and pyronine stains. Varying the time intervals of therapy and sacrifice did not change the results. We conclude that under these conditions neither vitamin B_{12} nor aureomycin exerted either a protective action to the liver or a stimulus to liver protein regeneration.

Effect of ACTH, cortisone and adrenalectomy on the renal disease produced by anti-kidney serum in young rats. CAROLYN W. FORMAN AND W. E. EHRICH. *Cornell Univ. Med. College, New York City, and Graduate School of Medicine of Univ. of Pennsylvania, Philadelphia.*

The experiments to be reported were prompted by the clinical observation that ACTH and cortisone are of therapeutic value in the nephrotic syndrome, by experimental evidence that ACTH modifies the glomerular nephritis produced by injection of horse serum in rabbits (Rich *et al. Bull. Johns Hopkins Hosp.* 87: 549, 1950) and by the demonstration of Heymann and Land of the similarity of experimental renal disease in rats to the nephrotic syndrome in man (*Science*, 108: 448, 1948). Four-week old Long-Evans rats were given intravenous injections of rat anti-kidney serum produced in rabbits. Two groups of animals so treated were injected subcutaneously with ACTH and cortisone, respectively, throughout the course of observation. A third group was adrenalectomized prior to receiving anti-kidney serum. The clinical and pathological findings will be presented and their etiological and pathogenetic significance discussed.

Effect of 2,6-diaminopurine on the multiplication of the virus of Russian Encephalitis. CHARLOTTE FRIEND (introduced by C. P. RHOADS). *Sloan-Kettering Inst., New York City.*

The oncolytic effect of the virus of Russian Spring-Summer Encephalitis (RSSE) on several transplanted tumors in mice has been reported previously. The mice so treated eventually succumb to the virus infection. Therefore, a phase of

our program includes a search for agents with viricidal activity. Since it has been reported that 2,6-diaminopurine inhibits the multiplication of vaccinia virus *in vitro*, a preliminary study was made to test the effect of this compound on RSSE. The results of *in vitro* studies are presented herewith. RSSE multiplies readily in a Maitland type tissue culture medium, containing Tyrode's solution, horse serum and minced mouse tumor tissue. This method lends itself well to the assay of potential chemotherapeutic effects on the virus as well as the tissue. It was found that a 10^{-3} M concentration of the diaminopurine added to the culture containing minced mouse sarcoma 180 or mouse carcinoma 1025 as the tissue source, completely inhibited the multiplication of the virus. The growth of sarcoma 180 was somewhat affected by this concentration of the diaminopurine but this was not the case for carcinoma 1025. At concentrations of 10^{-5} M or less, the effect on the virus was not pronouced. The inhibitory action of the diaminopurine on viral multiplication could be completely blocked or reversed by adenine and partially blocked or reversed to a slight degree by guanine.

Inhibition of 17-ketosteroid excretion by *p*-(di-*n*-propyl sulfamyl)-benzoic acid ('Benemid'). LYTT I. GARDNER, JOHN F. CRIGLER AND CLAUDE J. MIGEON (introduced by EDWARDS A. PARK). *Dept. of Pediatrics, Johns Hopkins Univ. School of Medicine and Harriet Lane Home of Johns Hopkins Hosp., Baltimore, Md.*

P-(di-*n*-propyl sulfamyl)-benzoic acid ('Benemid') has been shown by others to inhibit a number of renal conjugative reactions. Since the urinary end-products of adrenal cortical steroid metabolism appear to be excreted as conjugates, the influence of 'Benemid' on the excretion of 17-ketosteroids (17-KS) and neutral reducing lipids (11-OCS) has been studied. 17-KS were measured by the *m*-dinitrobenzene reaction in ethanolic KOH solution. 11-OCS were measured by the reaction of phosphomolybdic acid with chloroform extracts which had been washed three times with 0.1 N NaOH and partitioned between benzene and water. Serial 24-hour urine collections were made on 2 normal, male adults. At the end of control periods 'Benemid' was given orally 0.5 gm. each 6 hours (2 gm/day). 17-KS excretion decreased by 50% beginning the first day of treatment and returned to normal when therapy was stopped (cf. similar action of caronamide, BISSELL *et al. Proc. Soc. Exper. Biol. & Med.* 72: 584, 1949). 11-OCS excretion showed no significant changes. Using a dose of 4 gm/day did not produce a greater decrease of 17-KS, nor did it alter 11-OCS from control values. This dissociation of effect between the structurally similar 17-KS and 11-OCS is reminiscent of the previously known fact that 'Benemid' inhibits *p*-aminosalicylic acid excretion but has no effect on acetylsalicylic acid excretion.

Antihemophilic factor: levels in canine and human plasmas, and following liver injury and Dicumarol. JOHN B. GRAHAM,* DAVID L. COLLINS, JR.,* IRA D. GODWIN* AND K. M. BRINKHOUS. *Dept. of Pathology, Univ. of North Carolina, Chapel Hill.*

The assay procedure for plasma antihemophilic factor (AHF) is based on the principle that the amount of prothrombin consumed in hemophilic blood is proportional to the amount of AHF (normal plasma) added (*Federation Proc.* 9: 330, 1950). By modification of the assay procedure, its sensitivity has been increased, and the quantity of AHF in the unknown sample can be expressed in per cent (control plasma equals 100%). A series of dilutions in duplicate of both unknown and control plasmas are tested with whole canine hemophilic blood; after 20 minutes under standard conditions, citrate is added and the residual prothrombin determined, using the modified 2-stage prothrombin test. AHF concentration of the test plasma is determined from the average ratio of unknown/control at the 70, 50, and 30% level of prothrombin utilization. With this test, as little as .0013 ml. of dog plasma or .0036 ml. of human plasma can be detected readily. Sensitivity is increased further by prolonging the reaction time. Dog plasma appears to contain several times as much AHF as human plasma. With acute liver injury (chloroform intoxication), no significant alterations in AHF could be detected, although the plasma was nearly devoid of fibrinogen and prothrombin. Transfusions with such plasmas were effective in correcting the clotting defect in canine hemophilia. Also, dogs made hypoprothrombinemic with dicumarol retained a full complement of plasma AHF.

Localization of the antigen responsible for the production of nephrotoxic serum within the isolated renal glomerulus. SEYMOUR A. GREENSPON* AND CECIL A. KRAKOWER. *Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago.*

Glomeruli have been shown to be the source of nephrotoxic antigen in the renal cortex of the dog (*Arch. Path.* 49: 291, 1950). In the present study, isolated glomeruli were sonically vibrated yielding at low voltage isolated cells, predominantly of visceral epithelial origin. At high voltage three fractions were obtained: a low speed centrifugate made up of clear refractile plates of parietal capsule and basement membrane optically free of cells, a high speed centrifugate, and a supernatant representing most of the components of all the cells of the glomerulus. By studying the anti-

genicity of low speed centrifugates with a high or low percentage of parietal capsules, it could be clearly shown that the latter were not antigenic. Isolated cells and all the cells of the glomerulus had approximately the same degree of antigenicity. By virtue of the distribution of DNA and PNA in the high voltage fractions, nuclei and probably cytoplasmic nucleoproteins could be excluded as bearers of antigen. Basement membrane was found to be twenty times more active antigenically than the cells in terms of wet weight and fifty times, on the basis of nitrogen content. Since one could not recognize morphologically or experimentally separate cellular cytoplasmic films which might be adherent to the basement membrane, the views is held that the nephrotoxic antigen is distributed throughout the basement membrane and that surface antigens are liberated with the separation of visceral epithelial and/or endothelial cells.

Effect of temperature and para-aminobenzoic acid on the growth and oxygen uptake of embryonate eggs. DONALD GREIFF* AND HENRY PINKERTON. *Depts. of Biology and Pathology, St. Louis Univ., St. Louis, Mo.*

Fertile eggs, after a preliminary incubation period of 8 days at 37.5° C., were found to use 0.31 cc. of oxygen/egg/hour, while the mean dry weight of the embryos was 62 mg. The eggs were then divided into groups and incubated at different temperatures. Five days later, the figures obtained were: at 34° C., 5.3 cc. of O_2 and 351 mg.; at 36°, 8.7 cc. and 551 mg.; at 37.5°, 10.8 cc. and 652 mg.; at 39°, 11.5 cc. and 710 mg.; at 40°, 10.8 cc. and 730 mg.; and at 41°, 7.5 cc. and 753 mg. For a group of eggs maintained throughout at 37.5° but receiving 6.6 mg. of paba on the eighth day, the figures were 12.2 cc. and 478 mg. Thus paba increases respiration in spite of retarding embryonic growth. The corrected respiratory rates (cc. O_2/egg/hr/gm. of dry embryo) were: eighth day at 37°, 5.1: 5 days later at 34°, 14.9; at 36°, 15.8; at 37.5°, 16.6; at 39°, 16.2; at 40°, 14.8; at 41°, 9.9; at 37.5° with paba, 25.6. These corrected figures are of value in determining the effect of the respiratory rate on the growth of rickettsiae and viruses in eggs. (Aided by a grant from the Natl. Insts. of Health.)

The form of binding of copper in serum. C. J. GUBLER,* M. E. LAHEY,* D. M. BROWN,* EMIL L. SMITH, G. E. CARTWRIGHT* AND M. M. WINTROBE. *Depts. of Medicine, Pediatrics and Biochemistry, Univ. of Utah, College of Medicine, Salt Lake City.*

Serum copper, total serum protein and electrophoretic analyses of serum have been made in normal human subjects and in patients with infections, leukemia and pregnancy. Studies have also been included on normal rats and on rats with experimentally induced inflammation. A striking correlation was found between variations of serum copper and α_2-globulin levels. Following experimentally induced inflammation in rats the rise in serum copper was accompanied by a corresponding rise in α_2-globulin. The correlation between copper and all other serum protein components was very poor. Total globulin showed a fair correlation with copper. Serum copper, even when elevated, will not react directly with sodium diethyldithiocarbamate at slightly alkaline pH, unless first liberated from protein by acid. However, after oral or intravenous administration of copper or *in vitro* addition of copper to serum, all of the copper in excess of the level previously present in the serum will react directly with carbamate. Serum copper is not transferred to red cells when incubated with them whereas *in vivo* or *in vitro* added copper transfers rapidly. These findings confirm those of Cohn *et al.* (*J. Am. Chem. Soc.* 68: 459, 1946), and Holmberg and Laurell (*Acta Chem. Scand.* 1: 847, 944, 1947) that serum copper is combined with an α-globulin and further suggest that this is an α_2-globulin. The loosely bound transport copper is probably combined in a different manner.

The site of α-estradiol inhibition of tissue respiration. MARION A. GUIDRY,* ALBERT SEGALOFF AND AARON M. ALTSCHUL.* *Depts. of Chemistry and Medicine, Tulane Univ., and Endocrine Research Lab., Alton Ochsner Med. Fndn., New Orleans, La.*

The respiration of rat liver mince made in phosphate buffer containing 1% nicotinamide was measured. Substrates of three different types were added to this preparation; lactate, glutamate and glycerophosphate whose oxidations are linked with cytochrome c through diphosphopyridine nucleotide, isocitrate, linked through triphosphopyridine nucleotide, and succinate whose oxidation is not coenzyme linked. In every case addition of substrate increased the respiration of the mince. This increased respiration, for substrates whose oxidation required either of the phosphopyridine nucleotides, was inhibited by α-estradiol at a concentration of 5×10^{-5} M. Other estrogens such as estrone, α-estradiol-17-propionate, α-estradiol-3-trimethylacetate, α-estradiol-17-hemisuccinate and α-estradiol-3,17-disuccinate also inhibited this respiration. The increased respiration, when succinate was the substrate, was not inhibited. This suggests that a component of the coenzyme systems is inhibited by the compounds listed.

Latent period of renal disease in rats induced by nephrotoxic duck serum. DONALD B. HACKEL AND WALTER HEYMANN (introduced by THOMAS D. KINNEY). *Depts. of Pathology and Pediatrics, Western Reserve Univ. School of Medicine, and*

Dept. of Pathology, Cleveland City Hosp., Cleveland, Ohio.

A latent period of 5–21 days was noted in 28 of 31 Long-Evans rats that were injected with anti-kidney serum obtained from 7 different ducks. A latent period was not noted when anti-kidney serum was obtained from rabbits. All of the rats injected with duck serum showed histological and blood chemical changes similar to the rats injected with rabbit serum, with albuminuria and an initial azotemia, marked hyperlipemia and hypoproteinemia. The duck serum, however, resulted in more severe glomerular lesions and over 50% of the rats developed progressive hypertension and azotemia or died of uremia. With serum obtained from rabbits, only 3% of Long-Evans and 11% of Whelan rats showed a similar course.

Mathematical relationships of possible significance in the study of human leukemia. P. F. Hahn and Norbert Wiener. *Cancer Research Labs., Meharry Med. College, Nashville, Tenn., and Dept. of Mathematics, Massachusetts Inst. of Technology, Cambridge.*

Many feel that the leukemic process involves a massive proliferation of leukopoietic tissue arising from a single focus of pathological cell or cells and that the effect of irradiation is one of the result of ionization effects directed toward the aberrant stem cell process. If such were the case, in view of the short life span of the leukocyte, one should expect a precipitous fall in the total leukocyte count very soon following the irradiation process. Usually from 3–4 weeks are required for the full effect of radiation to be shown. It has been noted that the fall in leukocyte count following therapy usually takes place according to a logarithmic function suggesting that the effect of therapy is one following a first order reaction. Furthermore during relapse the increase in the blood leukocyte level also in most instances approaches that of logarithmic function and there may often be a matter of 3 or 4 months involved in the progression from normal to the extremely elevated leukocyte levels. The life span of the white cell in the peripheral circulation is probably only a few days. Thus from a consideration of the time constants involved, it seems highly unlikely that the relapse phase of the leukemic process is simply one of a disturbance between the balance of formation and removal of the white cells from the pheripheral circulation. It would seem to be suggested that there was an active alteration in the 'breakdown and fed back' process concerned with the normal rate of delivery and/or maturation of immature leukocytes.

Carditis and pulmonary arteritis in monkeys. Tom R. Hamilton and Jerome T. Syverton. *Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minn.*

Comparative studies are under way to assess the significance of the histopathological changes in the tissues from the cardiovascular system of monkeys which had been kept under natural laboratory conditions, or had been subjected to experimental manipulation inclusive of the purposeful production of disease. The hearts from 216 monkeys were examined. From each heart sections were studied from seven sites selected by Gross, Anatopol and Sacks (*Arch. Path.* 25: 840, 1930; Hamilton, Dascomb and Syverton. *Federation Proc.* 9: 332, 1950) as the most likely areas of involvement in rheumatic disease. The results made it known that carditis occurred *a*) in 21% of 38 'normal' uninoculated controls, *b*) in 66 % of 75 animals that had undergone 'mock' operation, *c*) in 80% of 46 animals with known poliomyelitic or Coxsackie infection, and *d*) in 85% of the monkeys employed in experimental studies which included the injection of streptococci, and/or foreign protein. Thus, the results made it apparent that myocardial changes were present commonly in monkeys from all categories. Moreover, qualitative changes were observed. The occurrence of pulmonary arteritis in 17 of 63 animals that had survived intracerebal inoculation without giving evidence for infection by virus is of particular interest. Further descriptive material and the possible significance of these findings will be brought up for discussion. This research was conducted under Office of Naval Research Contract N6onr 24608, NR 130-762.)

Erythrocyte life span in small animals. Barbara A. Harrison, E. Langdon Burwell, and Clement A. Finch (introduced by S. W. Lippincott). *Dept. of Medicine, Univ. of Washington, Seattle.*

Radioactive iron (Fe^{55}) injected intravenously is rapidly synthesized by the bone marrow into hemoglobin. Tagged erythrocytes of approximately the same age may thus be obtained. When these radioactive erythrocytes are transfused to a normal animal, the circulating radioactivity will persist long beyond the period of red cell survival due to reutilization of the radioiron liberated in cell breakdown. This reutilization may be prevented by the injection of large amounts of non-radioactive iron into the recipient animals. The survival of a newly formed population of erythrocytes has been measured by this technique in animals. Life span is expressed as the number of days from the time when 50% of the radioactivity has disappeared from the circulation of the recipient. Possible errors arising from blood type incompatibility were excluded. The erythrocyte survival

in the dog was about 110 days, 50 days in rabbits and rats, and 25 days in mice.

Virucidal and bactericidal properties of B-propiolactone. F. W. HARTMAN, S. L. PIEPES* AND A. M. WALLBANK.* *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

Beta-propiolactone is one of the most promising of the 140 compounds screened to date in the search for a suitable virucide and bactericide in blood and plasma because of its effectiveness and relatively low toxicity. In the presence of 95% ACD plasma and whole blood, beta-propiolactone, in concentrations below 1500 mg/l., effectively inactivated a 1% suspension of brain tissue infected with the following viruses: New Jersey vesicular stomatitis, lymphocytic choriomeningitis, St. Louis encephalitis, rabies, Newcastle disease, SK strain of murine polio and encephalomyocarditis. In similar experiments, 2000 mg/l. or less sterilized ACD sheep serum containing approximately 5×10^7 organisms/ml. of the following bacteria: *A. aerogenes*, *E. coli*, *S. typhosa*, *M. aureus*, *Sar. Lutea* and *Ps. aeruginosa*. Since this compound breaks down rapidly on the alkaline side of neutrality, the pH of the suspending medium was lowered to 6.5 before the addition of microorganisms. To allow for complete degradation of B-propiolactone, the virus-compound mixtures were held at refrigerator temperature for 4 days before testing. Subsequent experiments showed that at 6–10° C. 12–24 hours were adequate, while at 24–27° C. 7–12 hours and at 37° C., 1–2 hours effectively destroyed the viruses. A decrease in the concentration of virus will reduce the amount of Betaprone necessary for sterilization.

Fatty cysts of liver: I. Discharge of intracystic lipid into bile canaliculi and sinusoids in choline-deficient rats: the etiological role of ruptured cysts in cirrhosis. W. STANLEY HARTROFT. *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Ontario, Canada.*

Livers of choline-deficient rats undergo a series of changes which culminate in cirrhosis. Initially, fat accumulates in *intracellular* form, but some becomes *extracellular* since it is released by rupture of membranes of adjacent cells which then form cysts filled with fat (*lipodiastaemata;* W. STANLEY HARTROFT. *Anat. Rec.* 106: 61, 1950). Extension of this process involves other cells and cysts so that as many as 60 nuclei may be counted in a cyst-wall. The larger a cyst, the more numerous are potential sites for rupture of the thin membranes between its lumen and those of sinusoids and bile canaliculi which cover it. Tears in partitions between lumina of cysts and adjacent canaliculi allow fat from the cyst to enter the biliary tree; tears involving sinusoids permit fat globules to enter the circulating blood with production of an intermittent type of fat embolism on a small scale. The cumulative effect of repeated fat emboli of hepatic origin on heart, lung and kidney will be demonstrated. Chains of ruptured cysts shrivel and their atrophic parenchymal cells and supporting stroma become compressed into annular bands by compensatory proliferation of periportal parenchyma. Cysts which communicate with bile canaliculi may persist as bile passages. Fibrous trabeculae of this type of cirrhosis thus consist of little more than atrophic remnants of ruptured cysts. (Supported by the Natl. Cancer Inst. of Canada.)

Further purification of embryonic growth-promoting substance for chick fibroblasts. GEORGE M. HASS, ALBERT SCHWEITZER* AND HELEN BOSCIA.* *Rush Dept. of Pathology, Presbyterian Hosp., and Univ. of Illinois College of Medicine, Chicago.*

Additional quantitative chemical fractionation and assay by tissue culture methods of the fibroblastic growth-promoting factor in chick embryonic extracts have been done. The factor appears in the 'cytoplasmic' rather than the 'nuclear' fraction of extracts. It is non-dialyzable and is insoluble in distilled water. The water-insoluble precipitate partly redissolves upon addition of Tyrode's solution. The soluble fraction of the precipitate contains 80% of the activity of the initial crude embryonic extract. The factor is also precipitated from dilute salt solution by addition of four volumes of acetone or ethanol. After exhaustive extraction of this precipitate and drying in vacuo, a white powder which retains about 60% of the activity of the crude extract is obtained. By dissolving the soluble part of this powder in diminishing quantities of Tyrode's medium, solutions which have greater activity than any crude extracts were prepared. It was calculated that the factor induced fibroblasts to divide twice each 24 hours. Denaturation, precipitation and removal of proteins in potent extracts with chloroform-octyl alcohol has given further purification. However, no active fraction, entirely free from protein or nucleic acids, as determined by the Folin Reagent and ultraviolet spectrophotometry, respectively, has been obtained.

Early development of anatomical and blood chemistry changes in nephrotoxic renal disease in rats. WALTER HEYMANN AND DONALD B. HACKEL (introduced by THOMAS D. KINNEY). *Depts. of Pathology and Pediatrics, Western Reserve Univ. School of Medicine and Dept. of Pathology, Cleveland City Hosp., Cleveland Ohio.*

After the single intravenous injection of rats with nephrotoxic rabbit serum, the BUN may start to rise as early as one-half hour. Total lipids begin to increase after 2–4 hours, while blood cholesterol starts to rise after 6–8 hours. The serum

protein concentration begins to decrease after 8–24 hours. The loss of urinary proteins could not always explain the degree of hypoproteinemia noted within such a short period. The observation that total lipid and cholesterol values increase before hypoproteinemia is established provides evidence against the assumption that the hypoproteinemia is the cause for the nephrotic hyperlipemia. The glomeruli show histological changes as early as one-half hour after injection, which may in part account for the azotemia. The tubules show no abnormalities until 4 hours. After this interval, hyaline casts are often seen and hyaline droplet degeneration of the tubular cells is frequently marked. Fat in the tubular cells may be present as early as 8 hours, but is not commonly seen until after 24 hours. There is no quantitative correlation between the blood total lipid content and the fat in the tubular cells, but in no case is fat present without an elevated blood lipid leve'. No histological or chemical abnormalities are seen in a series of controls injected with inactive rabbit serum and killed at comparable intervals.

Mechanism of Columbia SK virus hemagglutination. BENI HORVATH* AND CLAUS W. JUNGEBLUT. *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.*

Attempts were made to standardize the hemagglutination phenomenon by controlling the quantities of the reacting substances and the composition of the ionic environment. Adsorption of the viral hemagglutinin on sheep red cells was studied as a function of time and temperature. In these tests the rate of adsorption did not seem to be the limiting factor for the rate of hemagglutination. The reversibility of both adsorption and agglutination was investigated and data were collected on the possible identity of the hemagglutinin with the infectious agent. The results of studies designed to determine the chemical nature of the cell receptor will be reported.

Reaction of the pulmonary artery to minute emboli of whole rabbit blood. ROBERT B. JENNINGS,* WILLIAM B. WARTMAN AND BRYAN HUDSON.* *Dept. of Pathology, Northwestern Univ. Med. School, Chicago, Ill.*

When emboli of whole rabbit blood measuring 0.5 mm. in average diameter were injected into the ear vein of healthy adult male rabbits, they either became adherent to the walls of the pulmonary arteries or impacted in them. In either case an acute arteritis accompanied by endothelial proliferation regularly occurred. This process was reversible in some instances but in others was followed by organization of the emboli which resulted in eccentric fibro-elastic thickening of the intima. Occasionally, fibrous adhesions stretched across the lumen forming intravascular bridges. Fatty degeneration, calcification, hemorrhage, and atheroma were absent. The lesions appeared to be self-limited and to undergo healing and there was no evidence that they were progressive.

Effects of radiation on lens. FRANK B. JOHNSON AND SOLBERT PERMUTT. (introduced by ISADORE GERSH). *Dept. of Anatomy, Univ. of Chicago, Chicago, Ill.*

Rabbit lens was fixed by freezing and drying and embedded in paraffin. A modified Hotchkiss procedure which reduces the extraction of important constituents was applied to sections. In addition, sections were stained for metachromasia with toluidine blue. With the modified Hotchkiss technique, in normal lens, the cement substance stains consistently and is readily visible. The lens fibers are only slightly stained. The capsule and epithelium stain readily. After staining with acqueous toluidine blue, the cement substance, the capsule, and all nuclei are metachromatic; the fibers are orthochromatic. Identical methods were applied to frozen-dried rabbit lens in which grossly visible cataracts had previously been induced by radiation. With the modified Hotchkiss technique, the epithelium stains much more vividly than normal, while the cement substance stains less intensely and is thinner than normal. The lens fibers stain with variable intensity, and frequent areas of deep staining, two or three times the size of normal lens fibers, are seen; these probably represent degenerated fibers. With toluidine blue, metachromatic structures remain so; in addition, the degenerated areas, described above, are metachromatic. Morphologically the cataractous lens reveals thickening of the capsul and conspicuous distortion, with swelling, of the lens fibers. There is an increase in ice-crystal artifact in the cytoplasm of lens fibers and in all nuclei. This is presumptive evidence for dilution of cellular constituents by water.

Protective influence of pregnancy on experimental necrotizing arteritis in dogs. CHESTER K. JONES* AND RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans.*

The sequence of feeding a 'standard high fat diet' for 8 weeks or longer then inducing 'standard renal insufficiency' resulted in 'typical arterial lesions' in 14 of 14 dogs. The accidental observation that one dog (not included in the above summary) was pregnant and failed to show typical arterial lesions suggested additional experiments to determine the influence of pregnancy. Since the gestation period of dogs (63 days) closely parallels the time necessary for a satisfactory experiment, the first problem was to obtain female dogs as soon after conception as possible. Attempts to mate the

dogs under kennel conditions were unsuccessful. Our efforts to date have yielded 3 additional dogs in an early enough stage of pregnancy to be of experimental value. None of these three dogs fed the standard high fat diet had typical arterial lesions when they died in 'uremia' after the induction of standard renal insufficiency. These limited observations indicate that something connected with the pregnant state affords protection against the typical arterial lesion which are essentially examples of typical 'collagen disease' lesions. (Aided by grants from The Life Insurance Med. Research Fund and from the U. S. Public Health Service.)

Experimental production of rheumatic-like cardiac lesions in the guinea pig. RUSSELL S. JONES (introduced by DOUGLAS H. SPRUNT). *Div. of Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis.*

The idea that altered collagen is concerned in the production of rheumatic fever has been held by a number of workers. The experimental work of Cavelti is particularly of interest. On the basis of this and other work, we tried various types of collagen altered by such agents as acetic acid, urea, phenylhydiazine, salicyladehyde, periodic acid, and gluconic acid. With these preparations, we were able to produce the following cardiac valvular lesions: basophilic edema, fibroblastic proliferation, collagen formation, endothelial aggregates along the line-of-contact, and areas of necrosis. Groups of myocytes and mononuclear leukocytes occurred beneath the ventricular endocardium and about arteries with altered media. The presence or absence of vitamin C in the diet did not influence the incidence or severity of these lesions to any extent. In order to see if it was the altered collagen which produced the lesion or some other factor, we have tried a number of other substances and find that similar lesions can be caused by numerous bacterial toxins. We are now in the progress of exploring the specificity of these rheumatic-like cardiovascular lesions.

Inter-relationship of copper and choline when added to a choline deficient diet. N. KAUFMAN,* J. A. CARTAYA,* D. M. HEGSTED AND THOMAS D. KINNEY. *Dept. of Nutrition, Harvard School of Public Health and Depts. of Pathology, Western Reserve Univ. School of Medicine and Cleveland City Hosp., Cleveland, Ohio.*

Male albino rats were fed diets low in protein but adequate in all minerals and vitamins and supplemented with varying quantities of choline. In addition, these animals were fed moderately toxic doses of copper. It was found that the addition of choline increased the detrimental effect of the diet as measured by weight loss. Choline also increased the level of liver copper. It is concluded that choline either increases the absorption and/or the deposition of copper or inhibits its excretion.

Characterization of the vitamin A-like 'lard factor'. HANS KAUNITZ AND CHARLES A. SLANETZ* (assisted by RUTH ELLEN JOHNSON*). *Depts. of Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.*

In previous work, it has been demonstrated that a molecular distillate of lard (215° C. and 10^{-3} mm. Hg; Distillation Products Industries) contains practically none of any known form of vitamin A but has vitamin A activity (*J. Nutrition* 42: 375, 1950). Using a purified diet containing rancid lard for rat bio-assays (*Proc. Soc. Exper. Biol. & Med.* 75: 322, 1950), the activity of 1 gram of distillate corresponded to 60–90 units of vitamin A palmitate (Hoffman-La Roche) and that of undistilled lard, to about 4 units. Considering the concentration by distillation, this demonstrates heat resistance in the absence of oxygen. Mild saponification under N_2 effects loss of activity in the unsaponified and fatty acid fraction. Extraction with acetone resulted in a fraction with two to three times the activity of the distillate. This concentrate, therefore, had an activity forty times that of undistilled lard. Hydrogenation also seemed to destroy the activity. Experiments with acid hydrolysis are as yet inconclusive. Examinations of beef fat, chicken fat, olive oil, Wesson oil, and sesame oil indicated, in bio-assays, activities of about 10 units per gram of chicken and beef fats, 3 units in olive oil, and practically none in sesame and Wesson oils. These activities seemed much higher than was expected from chemical assays (done with J. J. Beaver, Columbia University). Whether these factors are identical with the lard factor is being investigated. (Aided by grants from the Research Corp.)

Vitamin B activity of lard distillate. HANS KAUNITZ AND CHARLES A. SLANETZ* (assisted by RUTH ELLEN JOHNSON*). *Dept. of Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.*

Normal growth was observed when rats were kept on a purified diet containing, as fat source, 10% rancid lard and when all known fat and water soluble growth factors were fed separately. When all B factors were omitted, the substitution of a molecular distillate of lard (Distillation Products Industries) for the rancid lard resulted in somewhat lesser weight deficits after rats had been transferred to the deficient diets on weaning. These differences, although slight, were persistent and were especially noticeable after well comparable groups had been on the diets for 2–3 weeks. Definite differences were noted when the rats had been offered diets deficient in riboflavin only. In

this case, the differences in the average weight gains for the series on rancid lard and on lard distillate were highly significant. Experiments with thiamine-deficient animals are not as yet conclusive. Substitution of fresh butter for the rancid lard seemed to give little improvement when all B factors had been withdrawn. Whether these results indicate the presence in lard of B factors, particularly of riboflavin, or whether other properties of the lard are responsible for this effect remains to be seen. (Aided by grants from the Research Corp.)

Blood coagulation defect induced in rabbits by papain solutions injected intravenously. AARON KELLNER, THEODORE ROBERTSON* AND HOWARD O. MOTT.* *Dept. of Pathology, New York Hosp.-Cornell Med. Center, New York City.*

A 5% solution of crude papain injected intravenously into rabbits in amounts of 0.8–2.0 cc/kg. has regularly produced a blood coagulation defect which in many respects resembles that of hemophilia. The defect is characterized by a marked prolongation of the clotting time of whole blood which becomes apparent within 30 minutes after injection and lasts for 6–12 hours. Bleeding time and platelet count were not notably altered, and neither circulating anticoagulant nor abnormal amounts of anti-thrombin were demonstrable in the blood of the injected animals. The prolonged clotting time was not shortened by the addition of calcium, serum accelerator-globulin, purified fibrinogen or prothrombin, but it promptly reverted to normal upon the addition of thrombin or of thromboplastin (rabbit brain or viper venom). The clotting defect appeared to be due to a deficiency or inactivation of either thromboplastin or an essential thromboplastin precursor. Papain caused prolongation of the clotting time only when given *in vivo*; it has no such effect when added to rabbit blood in the test-tube. The factor in the papain solution responsible for producing the clotting defect appeared to be associated with the solution's proteolytic activity, for heat-inactivation of the proteolytic enzyme also abolished the effect on blood coagulation, and highly purified papain solutions having concentrated proteolytic activity were still capable of inducing the coagulation defect. Trypsin solutions, on the other hand, did not produce a comparable coagulation defect upon intravenous injection.

Beta-propiolactone: its toxicity, degradation products and comparison with nitrogen mustard. A. R. KELLY* AND F. W. HARTMAN. *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

Beta-propiolactone (Betaprone), a virucide proposed by this laboratory as a candidate agent for sterilizing blood and plasma, has an i.v. mouse LD_{50} of 406 mg/kg. or $\frac{1}{100}$ that of the nitrogen mustard $CH_3N\ (CH_2CH_2Cl)_2 \cdot HCl$ (HN2). i.v. LD_{50}'s of the principal Betaprone degradation products in plasma are 2425 mg/kg. for hydracrylic acid and 1900 mg/kg. for sodium chloropropionate (2% formed). HN2 hydrolyzes to methyldiethanolamine (i.v. LD_{50} 1250 mg/kg., i.p. 2500). I.P. injections in 4 days of the methyldiethanolamine present in 75 human transfusion volumes of HN2 treated plasma showed no effects in mice. Both Betaprone and HN2 react with plasma proteins. Kelly *et al.* (1950) reported that under certain conditions, HN2 couples with plasma proteins to form leukopenic transformation products ('protein-imine'). Toxicity due to Betaprone combination with protein has not yet been demonstrated. Animals were transfused with plasma treated with HN2 and Betaprone at virucidal concentrations and effects evaluated on the following: growth curve, bleeding time, clotting time, prothrombin time, platelet count, C.B.C., color index, A/G ratio, serum bilirubin, urinalysis, bone marrow and tissue pathology. Immature mice received (in 15 days) the equivalent of 90 human transfusion volumes of Betaprone (1500 mg/l.) without significant effect. Findings were similar with plasma treated at 500 mg/l. HN2. Rabbits received the equivalent of 87 transfusion volumes in one month without effect. Transfusion studies with Betaprone treated blood are in progress. Ten and 20 cc. injections of treated blood (1500 mg/l.) in the human were uneventful.

Effect of multiple nitrogen mustard treated blood transfusions in animals. A. R. KELLY,* GEORGE H. MANGUN* AND F. W. HARTMAN. *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

In 1949 Hartman and Mangun proposed a virucidal nitrogen mustard, $CH_3\ (CH_2CH_2Cl)_2 \cdot HCl$ (HN2) as a sterilizing agent for blood and plasma. Kelly *et al.* (1950) reported that, under certain conditions, HN2 couples with plasma proteins to form transformation products with leukopenic activity. These substances, called 'protein-imine', necessitated modification of the sterilization method and extensive animal toxicity evaluation. Results with plasma have been previously reported and over 2000 human transfusions administered. This report deals with animal transfusions of HN2 treated blood. Homologous blood was used in all experiments and effects of HN2 (500 mg/l.) treatment evaluated on the following: Complete blood count, color index, growth curve, food intake, urinalysis, A/G ratio, bone marrow and tissue pathology. Immature mice received (in 12 days) the equivalent of 72 human transfusion volumes of treated blood (i.v. plus i.p. administration). Only a slight bone marrow stimulation resulted (statistically significant leukocytosis, neutrophilia and lymphocytosis). A

surgical transfusion-exsanguination technic was employed in mice allowing i.v. administration in 6 days of 22 transfusion volumes, without circulatory overload. Mild statistically significant leukopenia, neutropenia and lymphopenia followed removal of 50% of the blood volume and replacement with treated blood. This is apparently due to disintegration by HN2 of leukocytes in the transfused blood. Rabbits were transfused with their own HN2 treated blood (50 volumes in 4 months) with no effect except an early slight nonprogressive fall in white count. Multiple transfusions of pooled cross-matched blood in rabbits (44 transfusion volumes in 1½ months) were uneventful.

Effect of induced intimal proliferation on local deposition of lipids in arteries of rabbits with hypercholesterolemia. FRANK B. KELLY, JR.,* C. BRUCE TAYLOR,* GEORGE M. HASS AND JOHN M. CRAIG.* *Rush Dept. of Pathology, Presbyterian Hosp., and Univ. of Illinois College of Medicine, Chicago.*

Groups of juvenile (13) and senile (11) rabbits were used. A solution of cerebral lipids with 15% cholesterol was added to Purina Chow. Amount of food given was adjusted so each animal received .06 gm. cholesterol per pound of body weight per day. Blood cholesterol levels were determined at monthly intervals. After 22 weeks on the diet, three lesions were made by local freezing of the abdominal aorta and one lesion in each left renal and iliac artery using the procedure of Taylor *et al.* (*Arch. Path.* 49: 623, 1950). Animals were sacrificed at intervals from 2–52 weeks. Complete gross and microscopic studies were made. The vascular lesions were serially sectioned. Medial and intimal degenerative and regenerative changes subsequent to freezing followed the general pattern described by Taylor *et al.* Lipid material was deposited in lesions as placques on the surface of or beneath the initimal proliferation, or as lipophages in intima or media. Type and amount of lipid accumulation tended to be reduplicated in different aortic lesions of the same animal but varied considerably from one rabbit to another. This variation was apparently not related to the degree of spontaneous aortic atherosclerosis. Relation of lipid deposition to medial degeneration, blood cholesterol level, age of animal and age of lesions will be discussed.

State of hypersensitivity induced in rabbits to foreign serum proteins in Freund's adjuvant. SIDNEY D. KOBERNICK,* JOAN A. DE VRIES* AND ROBERT H. MORE. *Depts. of Pathology and Bacteriology, McGill Univ., Montreal, Quebec, Canada.*

It has been a popular belief that the state of hypersensitivity to foreign proteins injected in Freund's adjuvant is of the tuberculin type, or at least in some way qualitatively different from the type induced by protein antigens alone. Freund's adjuvant was used in 2 experiments to enhance the hypersensitivity of rabbits to horse serum and bovine gamma globulin. In the first, 2 injections were given 2 weeks apart each containing about one gm. of serum protein/kg. and the animals bled and skin tested one week. later. In the second, repeated injections of .1 gm. of the proteins were given at weekly intervals for 18 weeks. The animals were then bled and skin tested to graded dilutions of the serum antigens and to tuberculoprotein simultaneously. Where no necrosis of the skin occurred, the reactions to the serum antigens reached a maximum in 24 hours then receded rapidly. However, when necrosis did occur, the reactions tended to persist. Thus the persistence of reactions appeared to result from the necrosis rather than from the original antigen-antibody reaction. The tuberculin reactions were delayed for 48 hours, not reaching their maximum for 72–96 hours. The rabbits' serum showed high antibody titer and when injected into guinea pigs and appropriately challenged, induced fatal anaphylactic shock. Though the animals became sensitive to tuberculoprotein as well as to the serum antigens, the sensitivity to the serum antigens was not of the tuberculin type.

Enzymatic activity of liver and serum after experimental liver damage in rats. DIETER KOCH-WESER,* EMMANUEL FARBER,* J. DE LA HUERGA,* P. B. SZANTO* AND HANS POPPER. *Hektoen Inst. of Cook County Hosp. and Northwestern Univ., Chicago.*

Alterations of the enzymatic activity of serum and liver tissue (the latter determined chemically and partially histochemically) were compared with the degree of hepatocellular necrosis and other histologic changes in four types of acute experimental hepatic injury produced in rats: *a*) Uncomplicated fatty metamorphosis, produced by the administration of ethionine. *b*) Central necrosis, associated with fatty metamorphosis due to carbon tetrachloride. *c*) Extensive peripheral necrosis without fat accumulation due to allyl formate. *d*) Biliary stasis due to ligation of the common duct. The studies were performed in the acute stage, before regeneratory changes complicate the picture. In addition, a series of other chemical analyses on liver and serum were performed to ascertain the degree of hepatic functional derangement. Bronsulfalein retention, for instance, was most marked in ethionine intoxication and least marked after 5 days of common duct ligation. In liver and serum esterase activity (phenyl-benzoate) decreased and alkaline phosphatase activity (both determined biochemically)

increased parallel to the degree of liver cell damage as indicated by several histochemical methods. Changes in alkaline phosphatase and esterase activity in serum and liver was, as a rule, parallel except that the alkaline phosphatase activity in serum after common duct ligation increased the most. Diffuse fatty metamorphosis, which produced up to 30 gm. of total lipids/100 gm. wet liver tissue was not accompanied by changes in enzyme activity. Investigation on other enzymes are in progress.

Effect of plaster cast in tourniquet shock. SIMON KOLETSKY AND GORDON E. GUSTAFSON (introduced by ALAN R. MORITZ). *Inst. of Pathology, Western Reserve Med. School, Cleveland, Ohio.*

Tourniquet shock in rats could not be prevented by application of a plaster cast to the hind extremity immediately after removal of the tourniquet. The cast prevented significant swelling of the limb proper but did not eliminate edema. The fluid extravasate was shifted upward into the fascial planes between subcutaneous tissue and muscles of the abdominal wall. These sites cannot be restricted by external pressure. The effect of the cast was to reduce the rapidity rather than the ultimate magnitude of fluid loss. The slower rate was consistent with prolongation of survival time, even though mortality was not altered. At the time of death the magnitude of fluid loss in both case and control rats was comparable. Whether circulation in the injured leg was significantly altered by application of the cast is not known. The development of shock in cast rats was accompanied by hemoconcentration and rise in serum creatine, both of which occurred more gradually than in the control rats without casts.

Consideration of some variables other than hemoglobin dosage in production of hemoglobinuric nephrosis. JOSEPH J. LALICH. *Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.*

Observations in man and animals suggest that hemoglobin may exert an effect in the production of hemoglobinuric nephrosis which cannot be attributed solely to the amount of hemoglobin injected. Accordingly, to study the influence of extraneous factors other than dosage, the effect of aging of hemoglobin, conversion to methemoglobin with $NaNO_2$ and acidification of the hemoglobin solutions prior to intravenous injections were observed in rabbits. Because of the decided influence which the dose of injected hemoglobin may exert, all of the rabbits in this study received 1.0 gm/kg. of body weight. Five oat fed and 5 pellet fed rabbits were given injections of hemoglobin solutions kept up to 49 days at 4° C. No harmful symptoms or significant alterations in urine volume excretion were observed in these 10 animals. Hemoglobin acidified to pH 6.0 with one-tenth N HCl and reneutralized to pH 7.0 with Na_2CO_3 was injected into 10 rabbits. No significant effect on urine excreton was observed even in the 2 of 10 rabbits which died within 2 days after the injections. The pigment cast counts in the renal tubules of rabbits killed 7 or more days after injections were slightly higher in the oat fed than in the pellet fed group. Methemoglobin was prepared shortly before injections by mixing 5 ml. of 9 gm.% hemoglobin solution with 2 ml. of 1% $NaNO_2$. Following injections of 1.0 gm/kg. of methemoglobin into 4 rabbits no untoward symptoms or suppression of urine excretion were observed. Pigment cast counts in the renal tubules, however, were appreciably higher in the 2 oat fed than in the 2 pellet fed rabbits.

Effect of *p*-dimethylaminoazobenzene on the incorporation of labeled cystine into protein of the subcellular components of rat liver. NORMAN D. LEE,* NORMA M. MACRAE AND ROBERT H. WILLIAMS. *Dept. of Medicine, Univ. of Washington School of Medicine, Seattle.*

DL-cystine, labeled with S^{35}, was injected into normal Sprague-Dawley rats and into rats which had been fed *p*-dimethylaminoazobenzene for 8, 16 and 24 weeks. Eight hours later, the livers were removed and separated by differential centrifugation into the following fractions: nuclear, mitochondrial, microsomal and residual protein. The specific activities (counts/minute/micromole of sulfur) of the liver protein and aforementioned components were determined and evaluated in terms of the extent to which each fraction participated in S^{35} incorporation into liver protein. In the normal rat the rate of incorporation of S^{35} appeared to be similar in the mitochondrial and residual protein fractions. In the nuclear and microsomal fractions the S^{35} incorporation rates were less markedly similar and about half again as great as for the mitochondrial-residual protein fractions. At 8 weeks all fractions incorporated S^{35} at the same rate. At 16 weeks, the S^{35} incorporation activities of the four fractions appeared to have undergone a marked change; the rates of incorporation of S^{35} in the nuclear and residual protein fractions became similar and so did the rates for the mitochondrial-microsomal fractions. The former pair participated in S^{35} incorporation at an increasing rate over the remainder of the experimental period, whereas the latter pair of fractions showed a relative decrease in S^{35} incorporation during the 8 to 16-week interval and again an increasing rate, during the 16 to 24-week interval. The significance of these results will be discussed in detail.

High fat diet augments 'lipitis' and 'arteritis' due to allylamine. JOSEPH H. MCCORMICK,* CAR-

ROLL H. LIPPARD* AND RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ., School of Medicine, New Orleans.*

Eppinger, Mellon and Waters have previously shown that injections of allylamine are followed by curious necrotizing and proliferative changes in the fat and arteries of the mesentery and heart. Closely related amines did not produce this result. We have confirmed and extended these observations as follows: A control group of 15 dogs fed the regular kennel diet of table scraps and an experimental group of 8 dogs fed a specified high fat diet (with 43% of its caloric value derived from butter fat) were injected intravenously with 15 mg/kg. of allylamine on 3 consecutive days and sacrificed on the 7th day following the initial injection. All 8 of the experimental group were 'positive' while only 10 of the 15 'control' dogs had lesions. These results indicate that prefeeding a high fat diet influences the lesions produced by allylamine and constitute additional evidence for a relation between fat metabolism and vascular lesions. Allylamine injections afford a unique simple method of producing arterial lesions in dogs without the complicating factors of renal insufficiency or arterial hypertension. (Aided by grants from the Life Insurance Med. Research Fund and from the U. S. Public Health Service.)

Non-lipid, non-carborhydrate vacuolated cells in the healing skin wounds of cortisone-treated rats. J. F. A. MCMANUS, JAMES R. CASH, J. P. CARTER, E. M. ALRICH AND E. P. LEHMAN.* *Depts. of Pathology and Surgery, Univ. of Virginia School of Medicine and Hosp., Charlottesville.*

Twenty rats of the Wistar strain received midline abdominal wounds and were kept on a standard laboratory diet. Two days before the wounding the rats were given 40 mg/kg/day of cortisone. This was continued daily for the duration of the experiment. The animals were sacrificed at 2-day intervals up to the 12th day after wounding. Four equivalent sets of control were used consisting of adrenalectomized animals, ACTH treated, untreated and saline injections. The wounds were studied for tensile strength, and have been separately recorded. This report describes the appearance of vacuolated cells in the wounds of the cortisone-treated animals within 2 days after wounding. These cells increased progressively in number and appear characteristic in this series of the cortisone-treated animals. The cells were found in the neighborhood of the wound. As others have described the wound showed suppression of healing and decrease of fibroplasia. The contents of the vacuoles in the peculiar cells have been studied histochemically, and they do not consist of carbohydrates or unsaturated lipid, or of fat demonstrable with either Sudan IV or Sudan Black.

Chemical basis of the periodic acid-Schiff's reagent (PAS) method. J. F. A. MCMANUS AND CORNELIA HOCH-LIGETTI.* *Dept. of Pathology, Univ. of Va. School of Medicine, Charlottesville.*

Previous studies on the chemistry of the PAS method have included: *1*) acetylation and saponification of tissue sections (McManus and Cason), *2*) the action of pectinase, pectin-esterase and B-glucuronidase on tissue sections (McManus and Saunders), and *3*) the reversible blockage of pectinase action by acetylation (McManus and Cason, unpublished). The present communication describes the reversible blocking of pectin-esterase action by acetylation and the application of the PAS method, with and without acetylation, to chemically identified pectin, hyaluronic acid, heparin and mucopolysaccharides from ovarian cyst fluid. The results are described and discussed. (Aided by a grant from the Federal Security Agency, Public Health Service.)

Concentration of aureomycin in the intestine. LEON V. MCVAY,* LAREY EVANS* AND DOUGLAS H. SPRUNT. *Divs. of Medicine, Pathology and Bacteriology, Univ. of Tennessee College of Medicine and John Gaston Hosp., Memphis.*

In an effort to clarify the mode of action of aureomycin in intestinal infections and to determine its value as a preoperative agent in intestinal surgery, we investigated patients having cecostomies, transverse colostomies, or sigmoid colostomies. An attempt was made to correlate oral dosage, blood and intestinal aureomycin levels as well as alterations in the bacterial flora in various portions of the intestine. The following procedure was employed: after obtaining control specimens, each patient was given 250 mg. of aureomycin orally at 10:00 A.M., 2:00 P.M., 6:00 P.M. and 10:00 P.M. for 2 days; then, several days after the intestinal aureomycin had disappeared, this was repeated using 500 mg. of aureomycin; again, after the aureomycin level had been nil for several days, 750 mg. of aureomycin were administered four times a day for 2 days. Intestinal specimens were obtained at approximately the same period every 24 hours. While the range of levels was rather wide, all determinations were consistent in reflecting the rapid attainment and subsequent maintenance of significant intestinal levels of aureomycin. Each intestinal specimen was examined bacteriologically. Eight of the most common pathogenic organisms in peritonitis resulting from perforation of the intestinal tract are: *Escherichia coli*, *Streptococcus anaerobius*, *Bacteroides*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Clostridium perfringens*, and *Aerobacter aerogenes*. Of these organisms, only *Streptococcus faecalis* was not significantly reduced. This inves-

tigation emphasizes the value of aureomycin in intestinal disease.

Species specificity of thromboplastin; role of the co-thromboplastin reaction. F. D. MANN AND MARGARET HURN.* *Div. of Clinical Labs., Mayo Clinic, Rochester, Minn.*

It has long been recognized that mammalian thromboplastin is relatively inactive with respect to avian plasma. When thromboplastin prepared from rabbit brain was exposed to dilute (1:100) homologous serum its activity with respect to chicken plasma was greatly increased. The serum used had been aged at room temperature at least 24 hours and was shown to be essentially free of prothrombin. To produce the increased thromboplastic activity a definite period of exposure to the dilute serum was required (about 3 minutes) but no formation of thrombin was detectable. This is characteristic of the co-thromboplastin reaction. After treatment of the rabbit with dicumarol, which greatly decreases co-thromboplastin activity of both plasma and serum, the serum was virtually inert when tested as above. Since the specificity of thromboplastin with respect to the species studied was largely eliminated by allowing the co-thromboplastin reaction to occur, it is reasonable to suppose that the specificity largely concerns this part of the coagulation process, the phase most intimately concerned with thromboplastin.

Distribution of radioactive colloidal gold following intrapulmonary administration. GEORGE R. MENEELY, ROSS C. KORY,* STEWART H. AUERBACH* AND PAUL F. HAHN. *Radioisotope Unit, Thayer Veterans Administration Hosp., Depts. of Medicine and Pathology, Vanderbilt Univ. School of Medicine and Cancer Research Labs., Meharry Med. College, Nashville, Tenn.*

The distribution of radioactive colloidal gold instilled into one lung of anesthetized dogs was investigated. One-half ml. per pound of body weight of a distilled water dilution of standard therapeutic colloidal gold was placed in the trachea with the animal lying on its right side. Dogs were sacrificed after 1, 4, 6 and 15 days. Lungs, regional lymph nodes, liver and spleen were examined and samples taken for radioactivity assay and microscopic examination. The right lung exhibited high levels of radioactivity, and, early, the lymph nodes in the right hilum show moderate radioactivity. Very little of the colloid entered the left lung. Later, after 15 days, higher levels of radioactivity were found in the mediastinal lymph nodes. Very little of the colloid ever enters the blood stream as evidenced by negligible levels of radioactivity in the liver and spleen. Previous experience has shown that these organs effectively remove gold colloid from the blood by phagocytosis. In animals that received as much as one-half or one millicurie of radioactive gold per pound marked histological changes indicative of radiation effect were found in those tissues containing the higher levels of radioactivity. The nature of the distribution observed suggests that this route of administration might permit internal radiation of carcinoma of the lung which has spread to the regional nodes.

Crystallization of pyrexin, the pyrogenic factor of inflammatory exudates. VALY MENKIN. *Agnes Barr Chase Fndn. for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Pa.*

Evidence has been presented that in acid exudates, the presence of a pyrogenic factor, pyrexin, reasonably explains the mechanism of fever with inflammation. Present studies indicate that pyrexin, associated with the euglobulin fraction of acid exudates, can be crystallized out. When pyrexin has been obtained as described elsewhere (MENKIN, VALY. *Arch. Path.* 39:28, 1945; *Newer Concepts of Inflammation.* Springfield, Ill.: Thomas, 1950) it is suspended in a volume of distilled water equal in cubic centimeter to the weight in milligrams of the sample studied. An equal volume of saturated $(NH_4)_2SO_4$ is added. The mixture is dialyzed. The indiffusible material is thoroughly centrifuged. To the supernatant an equal volume of glacial acetic acid is added, and the mixture is placed in a refrigerator for several days to crystallize out. The crystals are rhomboid-like in appearance, but after dehydration they tend to be needle-like. These crystals, suspended in saline are highly pyrogenic within an hour or so. The average rise in temperature in 17 rabbits was 2.22° F. Various other controls do not show any rise in temperature. The supernatant mother liquor from which the crystals are extracted induced in 6 rabbits an average rise of 1.37° F. These facts suggest that pyrexin has been obtained in crystalline form. Amino nitrogen studies before and after hydrolysis suggest that this material is polypeptide in nature.

Effect of 2,6-diaminopurine on infection of Russian encephalitis in the mouse. ALICE E. MOORE AND CHARLOTTE FRIEND (introduced by C. P. RHOADS). *Sloan-Kettering Inst., New York City.*

The compound 2,6-diaminopurine, which has been shown to be utilized for the formation of polynucleotide guanine, has numerous biological effects. Thompson *et al.* found that it inhibited the multiplication of vaccinia virus *in vitro* but that it was ineffective *in vivo*. The compound completely inhibits proliferation of the virus of Russian encephalitis in a tumor tissue culture medium. Its effect on mice infected with this virus now have been established. The substance was administered to groups of mice intraperitone-

ally in amounts of 70 mg/kg. in 0.5 cc. saline. No toxicity occurred at this level. The virus of Russian encephalitis was inoculated intraperitoneally in dilutions of 10^{-5}, 10^{-6}, 10^{-7}. In one group of experiments mice received 2 or 3 injections of 2,6-diaminopurine before the virus was inoculated. The substance was administered daily thereafter until signs of disease appeared. These animals usually received a total of ten injections. Results of five experiments showed that of 189 control animals, 36 or 19% survived. In the treated group of 180 animals 83 or 46% survived. In another type of experiment the virus was inoculated first and injections of 2,6-diaminopurine begun 24 hours later and continued for 6 days. Of 118 controls, 19 or 16% survived, in the 120 treated animals 45 or 37.5% survived. If administration of the drug was delayed to 48 hours after the virus, no beneficial effect could be noted. The same results were obtained when the virus was inoculated subcutaneously but no effect was noted when it was given intracerebrally. The drug was shown to have no effect on infections with the following viruses: West Nile, Ilheus, Bunyamwera and the Lansing strain poliomyelitis.

Excretion of P^{32} in the bile of rabbits following intravenous administration. FERRIN B. MORELAND* AND BÉLA HALPERT. *Veterans Administration Hosp. and Depts. of Biochemistry and Pathology, Baylor Univ. College of Medicine, Houston, Tex.*

The rates of appearance in the bile and of disappearance from the blood serum of radioactive phosphorus were studied following intravenous administration of 2.5–3 μc. as phosphoric acid into adult male rabbits anesthetized with ether. Following the injection, bile was collected through a cannula tied into the common bile duct and samples of venous blood were taken at intervals. Two hours after the injection the contents of the gallbladder and urinary bladder and a sample of blood from the inferior vena cava were obtained. The P^{32} content of all samples was then determined. Bile from the common duct reached a maximum activity between 0 and 15 minutes (corrected for the lag of collection) following injection and contained less activity than the blood serum at the same time. The rate of decrease in the bile from the maximum value paralleled that of the serum. The activity of the serum was five to twenty-four times that of the bile. Bile from the gallbladder was intermediate in activity between the bile from the common duct at the maximum and at the end of 2 hours.

Effects of cortisone administered subcutaneously and orally to dogs. C. W. MUSHETT, C. C. PORTER* AND R. H. SILBER. *Merck Inst. for Therapeutic Research, Rahway, N. J.*

The route of administration of cortisone has been found to influence the effects produced by this drug. Daily subcutaneous treatment of 3 dogs with 10 mg/kg. for a period of 2 or 3 weeks brought about a profound polyuria and polydipsia. Each animal developed a slight anemia accompanied by an increased erythrocyte sedimentation rate. Red blood picture values gradually increased to normal or supranormal levels despite continued treatment. A transient leukocytosis was also observed. Eosinopenia was marked within 2 weeks. At autopsy the dogs showed enlargement of the liver and atrophy of the adrenal glands, thymus and lymph nodes. Histologic examination of the livers revealed that the parenchymal cells were markedly enlarged and contained dense deposits of glycogen, particularly in the periportal and midzonal regions. In contrast to the foregoing findings, none of 3 dogs given daily oral doses of 10 mg/kg. showed polyuria or polydipsia. Two showed slight decreases in values of the red cell picture, but leukocytosis was not observed. One animal developed eosinopenia. At autopsy the thymus and lymph nodes were smaller than normal but the weights of adrenal glands and livers were normal. Microscopic examination of liver sections revealed small groups of cells or isolated cells showing enlargement and excess glycogen. The extent of this change was relatively slight in comparison with that noted in dogs treated parenterally. No tendency toward increased coagulability of the blood was observed in dogs treated either subcutaneously or orally with cortisone.

Renal and other lesions in dogs and rats from intramuscular injection of neomycin. ARTHUR A. NELSON, JACK L. RADOMSKI* AND ERNEST C. HAGAN. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Twelve dogs were injected intramuscularly with neomycin at 20,000, 10,000 or 5000 U/kg/day, approximately 96, 48 or 24 mg/kg/day. The lowest level has been used in man, although generally in divided doses. All levels affected the dog kidney; the highest uniformly caused death in from 1–3 weeks, with enough renal tubular damage to account easily for death. Epithelial necrosis in the proximal convoluted tubules was outstanding. Blood non-protein nitrogen was elevated, and P.S.P. excretion depressed. Marked congestion of the liver, moderate bone marrow changes, and considerable irritation at injection sites also occurred. At 5000 U/kg/day, the animals were sacrificed after 1 month without apparent effect. Renal damage was slight, but consistently present. Increased granularity and occasional desquamation of epithelial cells were most evident. At 10,000 U/kg/day, effects were intermediate. Differences

between the lots of neomycin used (200 and 225 u/mg.) were minimal, if present. Additional dogs are now being injected at 5000 u/kg/day with a third lot, potency 208 u/mg. Rats were injected intramuscularly for 6 days at levels from 30,000 to 500 u/kg/day. Renal lesions were of the same general type as in dogs, and for a given u/kg. dose were of about the same severity.

Changes in plasma phospholipides following whole body x-irradiation. Richard A. Nevé and Cecil Entenman (introduced by Michael B. Shimkin). *Div. of Biology and Medicine, U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

Several species of animals have been subjected to single exposures of 250 kv. x-rays and the levels of the plasma phospholipids determined at several intervals. In rats that had received 750 r total body x-irradiation the plasma phospholipids levels increased slightly at the second or third post-irradiation day after which the levels decreased. When doses of 750, 1500, 2500 and 5000 r were given, marked increases were noted in the plasma phospholipid values 48 hours post-irradiation. The earliest detectable change after a large dose (2500 r) occurred 18–24 hours after irradiation. X-irradiation also produced increases in plasma phospholipid levels in dogs, guinea pigs, rabbits and mice. Significant changes were not found in plasma protein levels under the conditions of these experiments.

Limiting factors in the conversion of prothrombin to thrombin. Charles A. Owen, Jr.* and Jesse L. Bollman. *Div. of Clin. Labs., Mayo Clinic, and Div. of Exptl. Medicine, Mayo Fndn., Rochester, Minn.*

When the transformation of plasma prothrombin to thrombin is observed at short intervals, the evolution of thrombin is seen to follow a sigmoidal curve. This curve may be converted mathematically to a straight line, the slope of which then can be calculated readily. By this means it was found that the prothrombin conversion rate (slope) is a function of the degree of plasma dilution; that is, the greater the dilution, the slower the rate. This slowing is due to the dilution of factors in plasma which convert thrombin to thrombin, and and is independent of the amount of prothrombin present. The addition of purified prothrombin to diluted plasma does not alter the conversion rate (the amount of thrombin formed each second), but does allow more thrombin to be formed over longer periods. Methods which do not allow for the complete conversion of the prothrombin present measure primarily the conversion rate, rather than the prothrombin concentration. However, both the amount and convertibility of prothrombin are important in the coagulation process.

Hematologic changes associated with the anamnestic response in man. Sanford Pariser, Richard A. Zucker and Leo M. Meyer (introduced by Irving Graef). *Third (N.Y.U.) Med. Service, Goldwater Memorial Hosp., Welfare Island, New York City.*

Twelve adult schick-negative males were given single 1.0-cc. doses of fluid diphtheria toxoid. Serial studies of antitoxin levels, serum protein values, and peripheral blood and bone marrow smears were made. All subjects showed a significant rise in serum diphtheria antitoxin levels. Total protein, albumin and globulin values were normal. Electrophoretic analyses of the sera of 4 individuals revealed no change in gamma-globulin percentage. In 10 of the 12 subjects blood smears were marked by the appearance, usually on the 6th to 8th post-immunization days, of plasma cells and basophilic cells resembling plasma cells ('plasmacytoid cells'). In 4 persons the number of these cells was considered significant, ranging from 3.0–6.0%. Bone marrow differential counts were done before immunization and on the 7th and 14th days after immunization. These revealed an increase in plasma cells of 2.2–3.2% in 4 subjects on the 7th day. In addition, there were 1.0–2.2% plasmacytoid cells in 3 of these patients. No abnormalities were noted in other cellular components of peripheral blood or bone marrow. Comparison of antibody levels with hematologic findings indicate a possible correlation between the height of the antitoxin titers and the appearance of plasma cells. The 4 persons whose antitoxin response 2 weeks after immunization exceeded 150 u/ml. of serum were those with bone marrow plasmacytosis. Three of these 4 subjects had 3.0% or more plasma plus plasmacytoid cells in the peripheral blood.

Analysis of aortic 'arteriosclerosis' in 300 consecutive autopsies. Gwendolyne Peck,* Henry C. McGill* and Russell L. Holman. *Depts. of Pathology, Louisiana State Univ. School of Medicine, and Charity Hosp. of Louisiana, New Orleans.*

In recent years there has been a growing consciousness that 'arteriosclerosis' is a definite metabolic disease and not a consequence of the ageing of tissues. This newer concept implies that the process develops in repeated episodic fashion leaving cumulative 'scars' that remain clinically latent until occlusion without collateral circulation involves a vital area with resulting disease. The well recognized discrepancy between 'lesions' and 'disease' merely reflects the great 'factor of safety' that the cardiovascular system possesses and in no way invalidates the assumption that if there was no lesions, there would be no disease. Emphasis must be shifted to early, possibly re-

versible, lesions before real progress can be expected. Analysis of the lesions in the aortas of 300 consecutive autopsies from the viewpoint of this newer concept of pathogenesis revealed no lesions in 36 cases, 'single crop lesions' in 53 cases, 'double crop lesions' in 59 cases, and 'multiple crop lesions' in the remaining 152 cases. There was no real difference in the prevalence of arteriosclerosis related to race or sex. The earliest single crop lesions was observed at 1½ years. There was no person beyond the age of 7 years without at least one crop of lesions; yet one colored female reached the age of 78 without anatomic evidence of more than one crop. There is apparently an increase in the rate of appearance of 'multiple crop lesions' in the decade between 45 and 55 years. Other analyses will be presented.

Effects of cortisone on normal and ascorbic acid-deficient guinea pigs. CONRAD L. PIRANI, ROBERT C. STEPTO AND KENNETH SUTHERLAND (introduced by GRANVILLE A. BENNETT). *Army Med. Nutrition Lab. and Dept. of Pathology, Univ. of Illinois, College of Medicine, Chicago.*

Cortisone (4 mg/day) was administered for 21 days to normal (2.5 mg./day ascorbic acid) and to ascorbic acid-deficient guinea pigs to investigate its effect on the mesenchymal tissue changes of scurvy and to study the possible relationship of ascorbic acid to adrenocortical function. Pair-fed and fed *ad lib.* controls were included in the study. Ascorbic acid-deficient guinea pigs receiving cortisone ate 8% more and lost 7.8% less weight than their controls. The adrenal hypertrophy of scurvy as well as the concomitant exhaustion of sudanophilic and birefrigent material of the adrenal cortex were partially prevented. Adrenal cholesterol depletion was less pronounced while ascorbic acid depletion was not affected. Liver weight and glycogen concentration were restored to almost normal levels. The islets of Langerhans were hypertrophied. Cortisone moderately reduced the hemorrhagic manifestations of scurvy. The articular and osseous lesions were considerably reduced in severity and somewhat modified. The number of circulating eosinophils was reduced in the normal animals but was greater than that of the controls in the vitamin C-deficient guinea pigs. In the latter group a marked normoblastic response was also noted following cortisone. The lymphocytes were not significantly affected. It appears that cortisone, in the doses used, although correcting some of the metabolic disturbances of scurvy and modifying some of the pathologic changes, does not alter fundamentally the course of the disease and cannot prevent death.

Susceptibility and resistance to superinfection in brucellosis. A. D. POLLACK, E. H. KELLY, A. N. GORELICK, W. BRAUN AND J. VICTOR. *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*

Attempts were made to superimpose *Br. suis* infection on guinea pigs having brucellosis due to *Br. abortus*. Morphological and bacteriological findings were compared. Guinea pigs with brucellosis due to *Br. abortus* were inoculated with *Br. suis* at 2, 4, 6, 8, and 12 weeks after the primary infection. Autopsies were performed 5 weeks after inoculation with *Br. suis*. Brucellosis due to *Br. suis* was distinguishable grossly from that of *Br. abortus*. In the former there were characteristic enlargement and suppuration in the regional lymph nodes, nodular granulomatous splenitis and widespread specific visceral lesions. In contrast, *Br. abortus* lesions developed more slowly and did not attain the size, intensity and dissemination observed in *Br. suis* infection. Lesions of *Br. suis* were regularly observed in animals inoculated 2 weeks after infection with *Br. abortus*. Thereafter, morphological evidence of *Br. suis* infection diminished. Most of the animals having brucellosis due to *Br. abortus* for 4 weeks or more were refractory to *Br. suis*. In general, the morphological and bacteriological results were similar. Occasionally, anatomical evidence for superinfection was found in the absence of bacteriological confirmation. Experimental brucellosis due to *Br. abortus* produces resistance to *Br. suis* infection.

Protein intake and pulmonary tuberculosis in rats and hamsters. HERBERT L. RATCLIFFE. *Penrose Research Lab., Zoological Society, and Dept. of Pathology, Univ. of Pennsylvania School of Medicine, Philadelphia.*

The diets used in these experiments consisted of vitamin-free casein, cerelose, cottonseed oil, cellulose, and all minerals and vitamins known to be required for growth of rats and hamsters. The high protein diet for both species contained 40% casein. The low protein diet for rats contained 6 to 8% casein, on which stable subnormal weights were maintained. Hamsters required 8 to 10% casein to maintain stable subnormal weights. The animals were started on the experimental diets at about 8 weeks of age. Six weeks later they were infected by inhaling small numbers of virulent tubercle bacilli (Wells' techniques. *Am. J. Hyg.* 47: 1 and 11, 1948). The rats (Long-Evans strain) were infected by bovine bacilli (Ravenel strain), and the hamsters by human bacilli (H37 Rv strain). Under conditions of adequate nutrition (commercial diets) Long-Evans rats that inhaled bovine bacilli, and golden hamsters that inhaled human bacilli developed tubercles slowly and at highly uniform rates for about 12 weeks. In the present experiments rates of development of tubercles were not influenced by the diets during the

first 10 weeks after infection. After 16 weeks, however, initial tubercles were larger in both species that received 40% casein. Within this time, also, time, also, hamsters showed secondary spread of infection. This was more pronounced in animals fed 40% casein. It is concluded that, under the conditions of these experiments, tubercle formation is enhanced by a high protein diet.

Leukocytic functions in acute inflammation in man as modified by ACTH. J. W. REBUCK, R. W. SMITH* AND R. R. MARGULIS.* *Henry Ford Hosp., Detroit, Mich.*

Forty-two windows were studied as to cellular content at timed intervals in 12 patients prior and subsequent to administration of ACTH. Antigens used were diptheria toxoid, old tuberculin and egg-white. Control windows in healthy volunteers were characterized early by histiocytes and neutrophils. At 8–12 hours lymphocytes appeared followed by hypertrophying lymphocytes at 12–14 hours. Largely due to this hypertrophy a predominantly histiocytic picture resulted at 16–24 hours. Patients studied were suffering from varied ocular diseases, sarcoid, periarteritis nodosa, rheumatoid arthritis, congenital syphilis and aplastic anemia. Pretreatment inflammatory responses were necessary secondary controls, e.g. a pretreatment pyogenic exudate occurred after non-pyogenic antigen. The *most important response with therapy was a dropping out of the lymphocytes* at 8–12 hours and consequently of the hypertrophying lymphocytes at 12–14 hours. Because of this ear lymphocytic hiatus the depleted exudate consisted largely of neutrophils and histiocytes. Cells in sarcoid were an important exception, histiocytes at 5.75 hours were more dominant than control 24-hour stages. In two windows in which phagocytosis of India ink was studied, inhibition of phagocytosis occurred even among neutrophils in contrast to pretreatment controls. In an occasional patient lacking clinical response to ACTH, positive verdoperoxidase reactions normally found in lymphocytes, histiocytes and neutrophils in acute inflammation were sparse or lacking. In those windows in which the pretreatment response showed marked eosinophilic migration, upon treatment there was diminution of both eosinophils and lymphocytes in the windows.

Effects of a single whole-body exposure to the deuteron beam on the blood and tissues of the white mouse. PAUL D. ROSAHN, CORNELIUS A. TOBIAS* AND JOHN H. LAWRENCE.* *Radiation Lab. and Donner Lab. of Med. Physics, Univ. of California, Berkeley.*

White mice were exposed to a single whole body irradiation by the deuteron beam of the 184-inch cyclotron in doses equivalent to 460 r.e.p., 905 r.e.p., and 1800 r.e.p. Following this procedure, studies of the peripheral blood showed a prompt and significant reduction in the total white count, and in the absolute number of neutrophiles and lymphocytes. A slightly delayed but marked reduction in platelets also occurred. Surviving animals which received the smallest exposure showed a return of these blood elements to normal levels by the 18th day. Severe aplasia was noted in bone marrow. Lymph nodes and spleen showed focal cellular necrosis, macrophage proliferation and loss of cellular constituents. The blood and tissue changes after whole body exposure to the deuteron beam were proportional to the roentgen equivalent doses, and similar to the changes known to occur following x-radiation. Tissue studies revealed the same changes in these animals as the changes reported in animals exposed to x-ray or neutrons.

Nonhemolytic streptococci in relation to a milk-borne epidemic of influenza diagnostic cutaneous tests and specific treatment with thermal antibody. EDWARD C. ROSENOW. *Bacteriological Research, Longview Hosp., Cincinnati, Ohio.*

The results obtained from the use of special methods in a study of an epidemic of influenza in a large hospital for the insane is reported. A nonhemolytic streptococcus was isolated in parallel from the nasopharynx of persons stricken and from the pasteurized milk supply. The onset of the epidemic was abrupt and disappeared after the milk supply was pasteurized at 158° F. for 30 minutes instead of at the standard method of 142° F. for 30 minutes. The streptococcus on isolation from patient and the milk produced pneumonitis histologically similar to that following inoculation of influenza virus on cerebral and nasal inoculation, and pleuritis on intraperitoneal injection in high incidence and was agglutinated specifically in parallel by convalescent serum. Erythematous reactions to intradermal injection of 'respiratory' thermal antibody indicating streptococcal antigen were greatest and reactions to antigen indicating antibody least at the onset of the disease. The former on retestings diminished; the latter increased as recovery ensued. Subcutaneous therapeutic injection of thermal antibody prepared with heat and hydrogen peroxide caused a sharp drop in antigen and an increase in antibody in 24 hours and usually a corresponding improvement in symptoms. For reasons still obscure, such did not occur in persons receiving penicillin. It was found that the streptococcus from both patient and milk was far more resistant to penicillin, streptomycin and aureomycin than pneumococci and beta hemolytic streptococci which may be the reason why results of treatment with these antibiotics were far from satisfactory.

Relation of plasma to biliary phospholipids. FENTON SCHAFFNER,* MARVIN MEITUS,* J. DE LA

HUERGA,* D. F. MAGEE,* FREDERICK STEIGMANN AND HANS POPPER. *Hektoen Inst. of Cook County Hosp. and Dept. of Clin. Science, Univ. of Illinois, Chicago.*

To understand the physiology of obstructive jaundice, phospholipid excretion in hepatic bile was measured in 5 patients and 5 dogs by giving P^{32}. Specific activity of plasma and biliary acid-soluble phosphates and phospholipids were determined. In man, plasma phospholipid activity reaches a peak after 24 hours and then slowly declines. In human bile, the amount of P^{32} found incorporated in phospholipid was too small to be measured. In dogs where the radioisotope dose was fivefold greater, plasma phospholipids behaved similarly. The rate of incorporation of P^{32} into bile phospholipids was independent and of at a lower level than that in plasma phospholipids. The six hour sample was an exception in that the specific activity of phospholipids in bile exceeded that in blood. Bile phospholipids are probably formed independently of plasma phospholipids since they follow different rates of incorporation of P^{32}. Even human experiments show the lack of relation of bile to plasma phospholipids. The difference in behavior of phospholipids in blood and bile support the idea that phospholipids are synthesized separately and metabolized independently in different sites within the liver cell. Since the total phospholipid in bile is low compared to plasma, it is likely that in early obstructive jaundice the slight elevation of plasma phospholipids can be accounted for by regurgitation. The presence of obstruction would not alter the incorporation of P^{32} into plasma phospholipids. However, in cases with very high plasma phospholipids another explanation must be found.

Fatty cysts of liver. II. Reabsorption of intracystic lipid in choline-deficient rats restored to normal diets. E. A. SELLERS AND W. STANLEY HARTROFT, *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Ontario, Canada.*

Release of the *extracellular* lipid contained in fatty cysts by way of the biliary and vascular systems in animals *maintained* on a choline-deficient diet has been described by Hartroft (Abstract—*Federation Proc.* 10: 1951). When choline was restored to the diet of similar rats, a third, somewhat more physiological route has been observed, which in these animals functioned in addition to the first two. *Intracellular* fat disappeared within a few days, but *extracellular* fat in fatty cysts still persisted. Afer one week globules of intracellular fat reappeared in the cells surrounding the cysts and an unusual appearance was evident which suggested that diffusion (by some unknown mechanism) of fat from within cystic spaces into surrounding cells had occurred. Disappearance of intracellular fat resorbed from the lumina of small cysts was nearly completed before the same mechanism began in larger cysts. Thus the absorption of intracystic lipid was intermittent and was characterized by periods during which nearly all the parenchyma was devoid of stainable fat. In some instances the atrophy of the flattened cells of the walls of the cysts appeared to have been reversed so that they regained their original form—that of normal liver cells. (This work was supported by the National Cancer Institute of Canada).

Some effects of phenyl γ-(2-pyridyl) propyl ketone (EJA II) on the central nervous system. I. H. SLATER, J. F. O'LEARY AND D. E. LEARY (introduced by JAMES K. SCOTT). *Divs. of Pharmacology and Orthopaedic Surgery, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Evaluation of the action of a series of 2-substituted pyridine, quinoline and piperidine compounds has revealed that EJA II is a potent, short activing depressant of the central nervous system. The median effective dose for the loss of the righting reflex after intraperitoneal injection in mice is 60 ± 3.7 mg/kg. and 105 ± 25 mg/kg. in rats. In rabbits, the intravenous head drop dose was 28–49 mg/kg. after 150 minutes; recovery was complete and the head drop dose was not decreased. Three or four such determinations in one day indicate a rapid removal of the drug from the body. During the brief period of maximal activity the compound protects mice from the extensor phase of maximal electric shock pattern (Median Effective Dose, 19 ± 2.2 mg/kg.). Although EJA II modifies the seizure pattern after metrazol given intraperitoneally and may prolong the survival time, the animals do not recover from two lethal doses of metrazol when given non-hypnotic doses of EJA II. Intravenous titration studies with metrazol in mice show that the drug does not elevate the convulsive threshold although an increase in the lethal dose usually occurs. There is no protection against strychnine. These findings suggest that the preponderant level of action is high in the cerebrospinal axis. Studies of the effect of EJA II on the reflex activity of the spinal cord in spinal and anesthetized cats show a selective depression of multisynaptic neuron arcs. After doses of 3 mg/kg. intravenously, the flexion and crossed extension reflexes are temporarily abolished without any change occurring in the knee jerk. No curariform action was noted in mice or in cats.

Human plasma fibrinogen levels in various pathological states. WILLIAM BROWN SMITH, LOUIS ROSENFELD AND GEORGE Y. SHINOWARA (introduced by EMMERICH VON HAAM). *Dept. of Pathology, Ohio State Univ., Columbus.*

Plasma fibrinogen levels were obtained by analysis of Fraction I of 975 specimens from 333 individuals. In the following groups the plasma fibrinogen levels were elevated: acute bacterial respiratory infections, 2–3 times normal in 3–7 days after onset; postoperative states, an average twofold increase in 4–5 days after surgery; dicumarolized patients, an average two- to three-fold rise by the 5th day following dicumarolization; acute pancreatitis, levels of 2–5 times normal; untreated malignancies, twice normal levels; radiologically treated malignancies, 3–4 times normal levels. On the other hand, fibrinogen levels of cases with viral infection began dropping during the second week of illness and remained low for as long as 7 months. In cases of Banti's syndrome the fibrinogen levels were slightly subnormal or normal. The normal mean value on adults was 0.26% with a standard deviation of ± 0.05%.

Effect of aureomycin on transplanted tumors. Boris Sokoloff and Walter H. Eddy (introduced by Leo Loeb). *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

E. Stokstad and T. Jukes reported that unpurified aureomycin exerts a growth promoting activity when added in small amounts to the rations of hogs, chickens and turkeys. The present investigation concerns the effect which purified aureomycin produces on transplanted tumors. Two types of tumors were investigated, August Rat Carcinoma 175-G, British brown breed of rats, and Rat Carcinoma R-13, black-white breed of rats. Altogether 190 rats were used for these experiments. Their average weight was 156 and 167 gm., respectively. When the rat bearers of 3-day-old transplants were given 1.6 mg/day/100 gm/weight, for 7 days, there was a definite increase in the size of tumor in the treated animals. At the end of three weeks of the treatment, the average size of tumor (20 rats) was 7.3 $cm.^3$, while the average control tumor was 3.9 $cm.^3$. In the next series of experiments the rat bearers of 7-day transplants were given the same dose for 10 days with similar results. 9.6 $cm.^3$ for the treated animals, and 6.5 $cm.^3$ for the control (40 rats). When, however, a much larger dose was administered the reverse effect was obtained. When a large dose of aureomycin, 8.0 mg/day/100 gm/weight was given to the rat bearers of one-day transplants for 18 days, a number of complete regressions of the growth were observed and the average size of the tumors was even smaller: 3.7 cm^3 four weeks after transplantation.

Mechanism of mucin activity. L. Spero, B. A. Waisbren, E. J. Schantz, and J. Victor. *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*

Addition of mucin to serum or heparinized blood or plasma decreased bactericidal action of these media. When plasma was treated with mucin and then centrifuged, the supernatant had no bactericidal activity while the precipitate still showed the activity of mucin when added to fresh serum. Addition of normal plasma to the supernatant resulted in a mixture with almost normal bactericidal activity. Therefore the effect of mucin was manifested even when an active principle was not present during the test. Furthermore, even after removing bactericidal substance from the blood, mucin still retained the capacity of removing bactericidal substances. The blood of guinea pigs which had been injected intraperitoneally with mucin lost its bactericidal activity, indicating a definite effect upon humoral mechanisms in the host. The blood of animals which were treated with mucin lost its bactericidal action but did not inhibit the bactericidal action of normal guinea pig blood. From the relation of mucin concentration to bactericidal action, simple dilution of the blood could not account for this effect. These observations indicated that mucin exerted its action by removing substances from the blood stream rather than by diffusing into and circulating in the blood stream. The combined data indicated that the change in lethal dose of bacteria which was brought about by intraperitoneal injection of mucin decreased the concentration of humoral factors concerned with host resistance.

Production of leukemia and cancer in adult rats fed paradimethylamino-azobenzene (butter yellow) shortly after weaning. Bernard Steinberg and Maryloo Spooner.* *Toledo Hosp. Inst. of Med. Research, Toledo, Ohio.*

Albino rats 6–14 weeks old were fed 3% butter yellow in a rice and carrot diet for 1–4 weeks. After the butter yellow was discontinued, some rats were placed on stock ration, others either on high fat or high protein diet. Of the 31 animals used, 5 developed either cancer or leukemia 13–17 months after withdrawal of the carcinogen. These experiments suggest the production of latent cellular injuries which become activated by the 'age factor.'

Ascorbic acid intake and the adrenal cortex. Robert C. Stepto, Conrad L. Pirani and C. Frank Consolazio (introduced by Granville A. Bennett). *Army Med. Nutrition Lab. and Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago.*

The effect of various levels of ascorbic acid intake on the histology and chemistry of the adrenal cortex was studied in 120 guinea pigs including pair-fed and fed *ad lib.* controls. Concomitantly, various factors, such as liver glycogen, serum sodium, blood lymphocytes and eosinophils, all of which may be affected by adrenocortical activity, were investigated. Representative animals from all groups were sacrificed at 7, 14 and 21

days from the beginning of the experiment. In the guinea pigs receiving no ascorbic acid there was a marked reduction in adrenal ascorbic acid and adrenal cholesterol accompanied by a fall in liver glycogen. Animals receiving 0.5 mg/day of ascorbic acid had a decrease in adrenal ascorbic acid but no decrease in adrenal cholesterol or liver glycogen. There was no appreciable change in adrenal ascorbic acid, adrenal cholesterol or liver glycogen in animals receiving 2.5 or 25.0 mg. ascorbic acid per day. No statistically significant differences were noted in the eosinophils, lymphocytes, or serum sodium. From these data it appears that as ascorbic acid content of the adrenal cortex is markedly reduced there is a simultaneous but less severe reduction in adrenal cholesterol. A compensatory hypertrophy of the adrenal cortex occurs only when the ratio of adrenal ascorbic acid to adrenal cholesterol exceeds 1:500 (normal 1:200). The hypertrophy is associated with an increase of adrenal proteins and with exhaustion of the histochemically demonstrable adreno-cortical steroids.

Relationship of complement to prothrombin activation. L. A. STERNBERGER AND FRANK MALTANER (introduced by G. DALLDORF). *Div. of Labs. and Research, New York State Dept. of Health, Albany.*

It has long been known that substances active in promoting or inhibiting blood coagulation also affect complement fixation (MALTANER. *Annual Report of the Division of Laboratories and Research, New York State Department of Health*, 1937, p. 13). The reverse demonstration of the effect of complement on blood coagulation is now reported. The effect of guinea pig complement on prothrombin conversion was measured by the stabilized thrombin method by determining the units of thrombin yielded per unit of time in defibrinated recalcified ethanol rabbit plasma with or without the addition of cephalin and/or barium sulphate treated plasma. It was found that with a mixture of one part of C′1 and one part of C′2 about twice as much prothrombin was activated per unit of time as with two parts of either component alone. Also, a mixture of one part of guinea pig serum lacking C′4 and one part of guinea pig serum deficient in C′3 activated about twice as much prothrombin per unit of time as two parts of either serum alone. Complement has also been largely removed from guinea pig serum by incubation with a sheep-erythrocyte-antibody system at 3° C. followed by centrifugation. The serum thus absorbed activated about half as much prothrombin per unit of time as serum absorbed with untreated sheep cells. It is suggested that the serum prothrombin conversion accelerator described by DeVries, Alexander and Goldstein (*Blood.* 4: 247, 1949). also functions as an accelerator of the immune hemolytic system. However, it cannot be decided from these experiments whether complement is essential for prothrombin activation.

Tumor immunity following regression of lymphosarcoma implants during riboflavan deficiency. H. C. STOERK, T. C. BIELINSKI* AND T. V. BUDZILOVICH.* *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Previously it was found in mice and rats that regression of lymphosarcoma implants during riboflavin deficiency is followed by an acquired resistance against re-inoculation with the same neoplasm. From the present experiments, it was seen that this tumor immunity was gradually lost over a period of about a year and that it rapidly diminished when marked loss of normal lymphoid tissue was produced by the administration of cortisone or of desoxypyridoxine. Also in lymphosarcoma-bearing rats deprived of riboflavin, the administration of cortisone or the induction of pyridoxine deficiency, with their attending depression of normal lymphoid tissue and of immune responses, prevented the expected tumor regression. Recently, a principle capable of destroying neoplastic cells was demonstrated by Kidd to be present in live lymphoid cells of animals immune to tumor grafts but not in the serum. Confirming Kidd's observations, we have found that cell suspensions from lymphoid tissue of immune rats exerted a cytotoxic effect on tumor cells. However, we have observed that standardized suspensions of tumor cells when incubated at 37° C. with serum from immune animals failed to produce takes when injected into non-immune animals. Suspensions of lymphoid tissue from immune animals heated at 42° C. for 15 minutes or unheated extracts of such tissues showed no cytotoxic effect. The injection of active suspensions of lymphoid cells from immune rats did not prevent takes in non-immune animals when the tumor cells were injected simultaneously but at a distant site.

Selective spastic occlusion of the renal vascular system produced with staphylococcal toxin. ALAN THAL (introduced by JOHN G. KIDD). *Dept. of Pathology, Cornell Univ. Med. College, New York City.*

In a study of the pathogenesis of symmetrical cortical necrosis of the kidneys, single small doses staphylococcal toxin (Wood-46 strain) were given to anesthetized rabbits in which the left kidney had been exposed by laparotomy. Within a few minutes the kidneys of the injected animals became first pale and swollen, then cyanotic, the swelling and cyanosis lasting for periods of several hours. During this time the renal vein alone of the visible veins remained in extreme spasm. Similar

changes were seen on clamping the renal artery of normal rabbits; hence it seemed possible that the toxin had produced a selective spastic occlusion of the renal vascular system. To learn more about this, India ink was injected into the aorta at the level of the diaphragm in a number of rabbits that had previously received the standard amount of staphylococcal toxin. Under these circumstances the kidneys alone of the abdominal viscera failed to blacken but instead maintained their cyanotic hue except for occasional flecks of black. During the period 40–120 minutes after injection of the toxin the renal blood flow, as determined in this way, was virtually nil in all animals. After 3–6 hours, however, the renal vessels in nearly half the animals filled rapidly and completely with the ink. All animals surviving 2–7 days showed normal renal blood flow, although in several cases examined histologically there was focal coagulation necrosis of renal tubular epithelium indicating previous ischemia.

Action of cortisone or its derivatives on an actively induced Arthus phenomenon in rabbits. F. W. TILLOTSON (introduced by A. R. MORITZ). *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.*

The Arthus phenomenon is the result of a localized antigen-antibody reaction and is most easily produced in the rabbit. The object of this experiment is to determine whether or not cortisone would alter this phenomenon. Young adult albino rabbits were divided into 3 groups of 8 animals per group. The first, or control group, was actively sensitized with horse serum (1.25 mg. protein intravenously per kg. 5 times per weeks for 2 weeks; then 2.50 mg. 5 times for one week) and a marked Arthus phenomenon was produced in 7 of the 8 animals. The second group was similarly sensitized but at the same time daily injections of cortisone were given (2 mg. per kg. per 24 hours for 25 doses). In this group the Arthus reaction was minimal or absent. The third group was similarly sensitized but cortisone was given only during the 3 days between the last series injection and the intracutaneous sensitizing injection. The reactions were minimal or absent—similar to the second group. Serum antibody determinations and histologic examination of tissues were also done.

Action of albumin on living cells of blood forming organs. EDNA H. TOMPKINS. *Cancer Research Inst. New England Deaconess Hosp., Boston, Mass.*

Comparative studies were made between freshly excised tissues suspended in solutions of albumin (crystalline, plasma, bovine, Armour) in Locke's solution, in Locke's solution alone and in homologous serum. These fluids were used both unstained and with the addition of supravital dyes. Erythrocytes from the human and mouse were studied. All other studies were made on tissues from the mouse. The observations were made with regular and phase microscopes. All measurements were made with the latter. Albumin increases definition and enhances detail in living cells. It intensifies supravital staining so that less stain is required to avoid toxic effects. Mitochondria stain normally with janus green. Staining with neutral red is darker than normal. Concentrations of albumin above 2% may be toxic. Lymphocytes and macrophages are more sensitive to toxic effects than the other cells studies. The diameters of lymphocytes from peripheral nodes are increased together with decrease in the nucleocytoplasmic ratios. Macrophages stain heavily, round, and exhibit broad bands of dense ectoplasm. Resting wandering cells of connective tissues are rounded by Locke's solution alone and this seems not to be modified by albumin. No changes are observed in fibroblasts and reticular cells other than increased nuclear detail. Suspended erythrocytes expand beyond their diameters in serum, and the undulating membranes become very pronounced. In the marrow there occur deeper staining of myelocytic granules and cessation of the migration of neutrophiles which is common in serum and is retained in Locke's solution alone.

Histologic study of neoplasms after heterologous transplantation. A. TOWBIN (introduced by E. VON HAAM). *Dept. of Pathology, Ohio State Univ., Columbus.*

It can be domonstrated histologically that tumor tissue undergoes a consistent series of changes after heterologous transplantation. The details of this process have been closely followed in transplantation studies of 100 human neoplasms using the anterior chamber technique. Three stages are involved in the establishment of a transplanted tumor: *Suspension stage.* During this period the tumor fragment remains loosely suspended in the aqueous humor. *Nidation stage.* This involves the organization and attachment of the tumor fragment to the iris or other surrounding tissue. Observed through the cornea, the transplant becomes vascularized, but shows no appreciable enlargement. *Stage of growth.* Outward growth from the edges of the transplant is evident histologically, the margin cells in the vacularized transplant showing mitoses and other evidence of proliferation. The pattern of development thus outlined provides criteria for a system of interpreting results in tumor transplantation experiments.

Inhibition of Tumor I of chickens by simultaneous infection with the malaria parasite *Plasmodium*

lophurae. WILLIAM TRAGER. *Rockefeller Inst., New York City.*

Chicks 2–3 weeks old were injected in the breast muscle with a freshly-prepared suspension of tissue of Chicken Tumor I. A portion of the chicks were inoculated intravenously on the same or on the following day with chick blood containing the erythrocytic stages of *P. lophurae*. In the chicks infected with malaria the growth of the tumor in the breast muscle was markedly less during the 7th–9th days following inoculation than in the control chicks. Some of the malarious chicks, when killed on the 7th day and autopsied, had no grossly visible tumor tissue whatever. The controls at this time had the breast musculature distended by large amounts of tumor and fluid. After the malarious chicks had recovered from their protozoan infection, they generally showed a rapid development of tumor, and eventually bore growths as large as those which had been earlier attained in the non-malarious chicks.

Effects of carbon tetrachloride and of starvation on cytoplasmic histochemistry of parenchymal cells of mouse livers. W. LANE WILLIAMS AND MARJORIE A. GREGORY.* *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Mice received one subcutaneous injection of 0.02–0.002 cc. of CCl_4, or were starved 1–7 days. Early injury (2–18 hours in CCl_4-treated; 36–48 hours of starvation) was demonstrated by *1*) liposis and positive alkaline phosphatase reactions *2*) progressive reduction in ribonucleic acid content (ribonuclease-basophilia method), glycogen, acidophilia (Hyden method), arginine (Serra-Thomas method), and tyrosine or histidine (diazo reaction). During early phases of injury these alterations were more evident in centrilobular zones. After 3 days of starvation all lobular zones were involved. Twenty-four hours after injection of CCl_4 the cytoplasm of irreversibly damaged central cells showed strong reactions for *1*) tyrosine-histidine, *2*) polysaccharides other than glycogen (periodic acid-Schiff's reagent), and *3*) acid-ophilia. Tinctorial intensities of reactions for polysaccharides and acidophilia were reduced by prior trypsin-hydrolysis (of tissue sections), indicating a protein-carbohydrate complex. Concomitantly nuclei of damaged central cells no longer reacted positively for desoxyribonucleic acid (Feulgen) and alkaline phosphatase, although the latter was positive in cytoplasm of central cells but not in parenchymal cells of other zones. CCl_4-induced injury was also characterized by liposis, and decreases in ribonucleic acid and glycogen in extracentral zones were subsequent necrosis did not occur. There fat was no longer demonstrable; and ribonucleic acid and glycogen returned to normal levels 3–6 days following injection of the polyhalogen.

Protective action of thyroid feeding and thyroxin injection against liver damage by carbon tetrachloride in mice. J. WALTER WILSON. *Biological Lab., Brown Univ., Providence, R. I.*

Mice were fed 0.4% defatted and desiccated thyroid, or injected intravenously with thyroxin, 4 μg. per gm. of body weight daily. After 3 days they received an injection of 0.2 cc. of a 40% solution of CCl_4 in sesame oil subcutaneously. This dose produces in controls a central necrosis of the liver involving 3–½ of the lobule. In thyroid treated mice, sacrificed on the second or third day after CCl_4, only a minimum of liver damage was found, at most a few necrotic cells and some leukocytes adjacent to the central veins. Thyroid feeding for one or two days was not adequate for protection. After prolonged feeding of thyroid in a semisynthetic diet, for 14 and 30 days, until the mice showed signs of thyrotoxicosis, the livers were still protected. Preliminary experiments have shown that desiccated thyroid also protects against liver damage by chloroform. This is contrary to the observations of MacIver (*Proc. Soc. Exper. Biol. & Med.* 45:201, 1940), who reported increased susceptibility to chloroform in the rat after thyroxin injections. Particularly after prolonged feeding of thyroid, the liver cells show a striking increase in cytoplasmic basophilia. Drabkin (*J. Biol. Chem.* 182: 335, 1950) has reported an increase in ribonucleic acid in the liver after thyroxin treatment. It is suggested that this may possibly play a role in the protective action against liver damage.

Effects of graded acute injuries upon net protein anabolism. R. W. WISSLER, E. P. BENDITT, L. E. FRAZIER,* C. H. STEFFEE AND F. L. JENNINGS.* *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.*

Groups of protein-depleted male albino rats were subjected on the same day to various acute injuries including fractures, total body x-ray, sterile abscesses, hemorrhage, thermal burns, chloroform injection and partial hepatectomy. Immediately after injury all groups were shifted to identical repletion rations containing high quality protein. All groups gained weight rapidly and showed substantial synthesis of carcass, liver, erythrocyte and serum proteins during the subsequent 14 days. When compared to the uninjured control group the rats receiving burns, sterile abscesses or total body x-ray showed a moderate reduction in weight gain and carcass protein in relation to the amount of protein consumed. Subsequent nitrogen balance experiments were performed utilizing 'protein-depleted,' 'repleting,' and normally nourished rats and employing burns

or sterile abscesses of varying degree. The normally nourished rats showed substantial increases in urinary nitrogen in the first 5 days after injury. This varied directly with the amount of injury. Some of these rats which received the greatest trauma were in negative nitrogen balance. The 'repleting' rats which were similarly injured, and were consuming almost the same quantities of an identical ration showed only a slight increase in urinary nitrogen in the same period. This was comparable to that shown by the depleted rats which were continued on a low protein diet. All of the 'repleting' rats were in strongly positive nitrogen balance. The results will be related to current ideas concerning the effect of trauma on protein metabolism.

Activation of trypanosomiasis in rhesus monkeys by cortisone. ABNER WOLF, ELVIN A. KABAT, ADA E. BEZER AND JAMES R. C. FONSECA. *Depts. of Pathology, Neurology, Bacteriology and Public Health, Columbia Univ. College of Physicians and Surgeons, and Neurological Inst., New York City.*

Macacus rhesus monkeys from India, obtained from a New York dealer in the winter of 1950, were found to be infected with *Trypanosoma vickersae* Brumpt 1909. Thirty-nine monkeys were used in testing the effect of cortisone upon experimental disseminated encephalomyelitis induced by inoculation of monkey brain and adjuvants. As an incidental finding, 12 of these developed lesions of trypanosomiasis, marked by encephalomyelitis and myocarditis, less often by myositis, neuritis, lymphadenitis and lipitis and rarely by hepatitis. Nine additional animals had a mild myocarditis, of which 6 had mild myositis and 2 mild encephalitis, resembling lesions of trypanosomiasis, but free of parasites. Of the 12 definite instances of trypanosomiasis, 1 severely and 1 mildly infected animal had received 6 injections of 5 mg. of cortisone subcutaneously per week, 1 moderately infected animal, 6 injections of 10 mg. weekly, 2 moderately and 2 mildly infected animals, 6 injections of 17.5 mg. weekly; 4 severely infected animals and 1 mildly infected, received 40 mg. daily. Of the 9 animals with suspicious lesions, in which trypanosomes were not demonstrated, all had received cortisone. Of 12 animals that received no cortisone, none showed trypanosomal lesions, although 2 had trypanosomes in their blood. It would seem that administration of cortisone activated a latent trypanosomal infection in these monkeys.

Spectrographic techniques for the quantitative analysis of human organs. ERNEST YEAGER,* I. L. SMITH,* NATHAN KAUFMAN,* FRANK HOVORKA* AND THOMAS D. KINNEY. *Depts. of Pathology and Chemistry, Western Reserve Univ., and Dept. of Pathology, Cleveland City Hosp., Cleveland, Ohio.*

Spectrographic techniques have been developed for the quantitative analysis of human organs for virtually all the common metallic elements. After dehydration to constant weight, the specimens are wet-ashed and the mineral elements converted to the sulfates or the nitrates if the former are insoluble. The resulting solutions are then excited by the high voltage a.c. spark source with the porous cup method of Feldman (*Anal. Chem.* 21: 1041, 1949). Sensitivities generally approaching 10^{-6} g/cc. of solution have been attained for many elements. The internal standard method has been used for quantitative analysis with cobalt as the control element. Working-curve shifts associated with large variations in the concentration of major constituents have been reduced by the use of specific solution standards which resemble the unknowns as closely as possible. Consideration has also been given to the introduction of inert gases into the analytical gap as a means for minimizing working-curve shifts as well as extending sensitivity. Accuracies of the order of 10% of the concentration have been obtained for a number of elements including some trace elements.

AMERICAN INSTITUTE OF NUTRITION

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(An asterisk following an author's name indicates "by invitation")*

Evaluation of nitrogen source materials by an intraperitoneal rat test. AARON ARNOLD AND JESSY R. SHEPHERD.* *Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

There is general agreement on applying the results obtained in evaluating protein hydrolysates by nitrogen balance studies in dogs given the preparations intravenously. There is not equal agreement, however, on applying the results obtained in evaluating such preparations by one of the oral rat-testing procedures. The latter do not distinguish between readily available free amino acids and poorly available amino acids bound as peptides, since under these conditions the peptides are fully subject to the action of the intestinal peptidases. The results to be presented are based upon intraperitoneal administration of the hydrolysates to rats, a mode of administration more nearly resembling the conditions of use. The preparations are administered at multiple levels for the 4 days of the test. Results obtained by this procedure indicate nitrogen utilizations of 48 to 63% for several commercially available preparations. These results demonstrate lower nitrogen retention than when essentially comparable preparations were tested orally in dogs (61 to 73%, SILBER AND PORTER, *J. Nutrition* 38: 155, 1949). The range of utilization by this rat method does compare favorably with that noted by the same investigators for nitrogen retention (40 to 57%) upon intravenous administration in the dogs. A comparison of results based upon urinary and fecal nitrogen excretion compared with results based upon urinary nitrogen excretion alone are also presented.

Utilization of injected carotene by vitamin A-deficient rats. J. G. BIERI AND R. P. SANDMAN (introduced by W. H. GRIFFITH). *Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Branch, Galveston.*

Tomarelli *et al.* (*Proc. Soc. Exper. Biol. & Med.* 63: 108, 1946) were the first to show that carotene dispersed in aqueous solution by Tween 80 was well utilized by rats when injected. The present study was undertaken to determine the minimum amount of carotene, in aqueous solution, which when injected would cure vitamin A deficiency and permit growth in rats. Male, weanling rats were placed on a purified, vitamin A-free diet. After 16 days the animals ceased to gain. On the 20th day they were evenly grouped on a weight basis. Purified B-carotene was dissolved in water with the aid of 1% chloroform and 20% Tween 40; α-tocopherol was added so that the supplement, in 0.1 ml., would contain 0.3 mg. of the antioxidant. The supplements were given daily for 16 days. The dosage and average daily weight gain per rat were: *group I*, 4.9 μg. intramuscularly, 3.9 gm.; *group II*, 10 μg. intramuscularly, 4.1 gm.; *group III*, 0.8 μg. orally, 1.9 gm.; *group IV*, 18 μg. orally, 3.9 gm. A fifth group, given a daily injection of 16 μg. carotene in cottonseed oil, lost an average of 0.8 gm. daily per rat for the same period.

Growth of rats fed heated or raw soybean oil meal supplemented with commercial trypsin. RAYMOND BORCHERS (introduced by C. P. BERG). *Dept. of Agricultural Chemistry, Univ. of Nebraska, Lincoln.*

Rats fed a 25% soybean oil meal ration (supplemented with methionine, salts and vitamins) gained 3.2 gm/day when the soybean oil meal was properly heated and 2.4 gm/day when the soybean oil meal was raw. When the ration was further supplemented with casein, whole liver, yeast, APF materials, etc., growth was enhanced by some of these but the respective growth difference between the heated and raw soybean oil meal rations persisted. When supplemented with commercial trypsin, growth was 3.4 gm/day on both heated and raw soybean oil meal rations. Fractionation of the commercial trypsin has established that the factor or factors which increase growth on raw soybean oil meal rations have no proteolytic activity.

Effect of adrenocorticotrophic hormone on amino acid content of blood and urine in normal individuals and patients with rheumatoid arthritis. A. L. BORDEN,* E. B. WALLRAFF,* E. C. BRODIE,* W. P. HOLBROOK,* D. F. HILL,* C. A. L. STEPHENS, JR.,* R. B. JOHNSON* AND A. R. KEMMERER. *Southwestern Clinic and Research Inst. and the Dept. of Nutrition, Univ. of Arizona, Tucson.*

Healthy university students were studied during the administration of ACTH for 7 days (40 mg. daily). They were kept on a controlled diet of adequate protein intake. During the period of study five 24-hour urine samples were collected on each individual. Tungstic acid filtrates were prepared from blood samples before and on the 3rd, 5th and 7th day of treatment. Patients with rheumatoid arthritis were metabolically controlled in hospitals and similarly treated. Microbiological assays for the amino acids reported were run on both the urine samples and the plasma filtrates. The results from the patients with rheumatoid arthritis show increased urinary excretion and slightly elevated plasma levels for some of the amino acids assayed. The changes in amino acids are strikingly less in the group of normal individuals although they follow the same general trend. Interestingly enough at the time the rheumatoid arthritis patients exhibited the changes in amino acid excretion there was a concomitant clinical response. The normal individuals did not experience the extreme sense of well-being, increased energy, and general stimulation so characteristically observed in rheumatoid arthritis patients similarly treated. The data here presented provide additional evidence of a correlation between the remission of rheumatoid arthritis produced by ACTH and amino acid metabolism.

Reactions of European and Indian cattle to wide temperature changes. SAMUEL BRODY, A. C. RAGSDALE,* H. H. KIBLER,* C. R. BLINCOE,* H. J. THOMPSON* AND D. M. WORSTELL.* *Dept. of Dairy Husbandry, Univ. of Missouri Agricultural Experiment Station, and U. S. Dept. of Agriculture, Columbia.*

Slides will illustrate the results obtained during 1948–50 on the effects of slowly rising and declining temperatures on large and small, lactating and dry, European and Indian (tropical) cows, and also on yearling heifers. Details are given in *Univ. Missouri Agr. Exper. Sta. Res. Bull.* 425, 433, 435, 436, 449, 450, 451, 460, 461, 464, and others in preparation. On plotting various reactions against rising environmental temperature, most curves showed sudden changes in slope at about 75°F. in European and about 90°F. in Indian cows. The decline in feed consumption at these temperatures presumably depressed the milk production, heat production, pulse rate, alkaline reserve (starvation acidosis), blood cholesterol and body weight. There were simultaneous increases in rectal temperature, respiration rate and blood creatinine. The increase in evaporative cooling began at lower temperatures. Indian cows lagged behind European cows by about 15°F. in all slope changes; but at environmental temperature 105°F., when the body-surface temperature also reached 105°F., and the rectal temperature reached 106–108°F., the curves of the Indian and European cows virtually coincided. The lag in critical temperature of the Indian cows is apparently associated with their greater heat-dissipating surface area per unit weight and nature of skin and hair. Less difference was observed between the European and Indian yearling heifers. The effects of lowering temperature to 0°F. were relatively slight.

Serum iron and hemoglobin values of 276 healthy women. MARILYN CHALOUPKA* AND RUTH M. LEVERTON. *Human Nutrition Lab., Agricultural Experiment Station, Lincoln, Nebr.*

Serum iron and hemoglobin values were determined on fasting venous blood of 276 healthy women who ranged in age from 17 to 86 years, were eating self-chosen diets, and were above average in socio-economic status. Serum iron was measured using the reagent ortho-phenanthroline, and hemoglobin was converted to oxyhemoglobin and measured photoelectrically. The serum iron and hemoglobin values were sorted into groups according to the age of the subjects. There were 52 women between the ages of 17 and 25 years; 35 between 30 and 40 years; 63 between 40 and 50 years; 64 between 50 and 60; 32 between 60 and 70; 22 between 70 and 80; and 8 between 80 and 86. The mean serum iron value for each age group mentioned above was 125, 119, 113, 122, 110, 95 and 93 μg. % respectively. Analysis of variance indicated that a significant difference existed between the mean serum iron levels of the different age groups ($F = 3.56$). The regression of serum iron on age was -0.370 which was statistically significant at the 1% level. The mean hemoglobin value for the women was 13.1 gm. %. Analysis of variance showed no significant difference among the mean hemoglobin values of the different age groups. No correlation was found between the serum iron and the hemoglobin values.

Corticosteroid determination with blue tetrazolium BT. CHIADAO CHEN AND HOWARD E. TEWELL, JR. (introduced by F. C. BING). *Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

Alpha: beta unsaturated-3-ketones of steroids other than corticosteroids were found to cause reduction of phosphomolybdic acid (see below). 3,3′-Dianisole bis-4,4′(3,5-diphenyl) tetrazolium chloride (blue tetrazolium BT) and alkaline copper were little affected by such ketones. Of the steroids tested, only corticosteroids with the primary alpha-ketol side chain caused a significant reduction of the blue tetrazolium BT. Procedure: To 1 ml. of methanol solution of steroid or methanol extract of urine, 1 ml. of 1 N NaOH in 80% methanol and 1 ml. of 1% blue tetrazolium BT in methanol are added. The solution is light shielded and kept at 25°C. Twenty minutes later the reac-

tion is stopped by 1 ml. of acid pyridine. Three ml. of methanol are added and the color read at 515 mμ with the Evelyn Colorimeter. A reagent blank is prepared from steroid-free methanol. For colored urinary extracts an additional blank is employed identical to the reagent blank except that 1 ml. of extract is added after the pyridine. The standard curve, utilizing desoxycorticosterone, follows Beer's law between 5 and 50 gamma per ml. and is reproducible over a wider range. The color is stable and the reagents are simple to prepare.

METHODS	OPTICAL DENSITIES FOR:		
	Progesterone *100 gamma*	Testosterone *100 gamma*	DOC *200 gamma*
Alkaline copper.....	.96	.94	.91
Phosphomolybdic acid...	.86	.87	.88
Blue tetrazolium BT.....	.97	.98	.90

Effect of basal diets on the response of rats to certain dietary non-ionic surface active agents. B. F. Chow, C. T. Ling,* L. Barrows* and J. M Burnett.* *Dept. of Biochemistry, School of Hygiene and Public Health, and the Dept. of Pathology, Johns Hopkins Med. School, Baltimore, Md.*

Sorbitan monostearate (Span 60), Polyoxyethylene sorbitan monostearate (Tween 60) and Polyoxyethylene monostearate (Myrj 52) were fed for 10 weeks to groups of 12 male and female weanling rats as 5% and 15% of a soybean meal diet (*A*): soybean meal 62%, sucrose 29.5%, corn oil 4.5%, and salt 4%, and the usual vitamin mixture. A similar group of rats was fed 5% Tween 60 in basal casein ration (*B*): casein 22%, sucrose 69%, corn oil 5%, salt IV 4%. Control groups were fed the two basal rations without the emulsifier. The addition of 5% Tween 60 to *B* caused diarrhea and a slight retardation of growth. No such effects followed the addition of any of the 3 emulsifiers to *A* either at 5% or 15% levels. Similarly, rats 1 year or older in groups of 12 each were fed *A* or *B* with and without the addition of 5% Tween 60. Throughout the 10 weeks of observation, the animals receiving the test substance in soybean meal did not develop diarrhea or lose weight, whereas those receiving emulsifier in the basal casein diet developed diarrhea. The results demonstrate that the emulsifiers tested can be fed to growing or aging rats at 5% or 15% levels in a normal diet without any clinical manifestation of toxicity throughout the experimental period. The gross and microscopical examination of these animals are also reported.

Amino acid requirements for the maintenance of nitrogen equilibrium in the rat. Helen Clark,* Pearl Swanson and S. P. Yang.* *Iowa State College, Ames.*

Nitrogen equilibrium ensues when a complete ration containing 4% of lactalbumin is fed to the normal adult rat. When the nitrogen content of the dietary mixture is limited quantitatively to only the essential amino acids provided by the lactalbumin in this diet, destruction of body tissue occurs as indicated by a marked negative nitrogen balance. When the nitrogen content of this latter ration is made isonitrogenous with the lactalbumin diet by the addition of the nonessential acids occurring therein, equilibrium is restored immediately. Furthermore, a linear relation exists between the quantity of nitrogen retained and the quantity of nonessential nitrogen added to the mixture of essential acids simulating lactalbumin, equilibrium being attained when 21 mg. of nonessential nitrogen/100 sq. cm. of body surface/day is fed. The nitrogen supplied by this mixture of nonessentia acids is not replaceable by an equivalent amount of essential amino acid nitrogen or of glutamic acid nitrogen. When the single acid supplements the essential nitrogen in the ration, the animals pass into acute negative balance. The omission of cystine from the mixture of nonessential acids results in catabolism of tissue. Its nitrogenous replacement with methionine permits equilibrium. Similarly, phenylalanine may replace tyrosine. Interestingly, the simultaneous isonitrogenous replacement of cystine and tyrosine with methionine and phenylalanine, respectively, induces marked positive balance. It is significant that nonessential nitrogen in the form of a single amino acid (glutamic) can supplement satisfactorily the mixture of essential nitrogen when it is modified thus.

Ascorbic acid and cholesterol studies in the rat during food restriction and subsequent protein rehabilitation. Ruth Clayton and Esther da Costa (introduced by Carl J. Koehn). *U. S. Army, Med. Nutrition Lab., Chicago, Ill.*

The average ascorbic acid of the adrenal of 184 male rats fed either a control, low calorie, high (60%) protein or modified carrot (2% protein) diet for 5 to 10 weeks was 0.23, 0.24, 0.21 and 0.21 mg/100 mg. of tissue and the average ascorbic acid of the whole blood was 0.9, 1.1, 1.5 and 0.7 mg/100 ml., respectively. Restricted animals were rehabilitated during ¾, 2, 3, 7, 35 and 70 days. After 2 or 3 days following 5 weeks of dietary restriction the ascorbic acid of the adrenal was one-third of the control value and blood ascorbic acid was increased almost three times. Average total adrenal cholesterol values of 3.1, 2.6, 4.4 and 4.3 mg/100 mg. of tissue and total serum cholesterol values of 55.2, 57.7, 63.7 and 65.0 mg/100 ml. were found in rats fed the control, high protein, low calorie and the carrot diet. After one week of re-

habilitation following 5 weeks on the carrot diet, total adrenal cholesterol was reduced to one half of the control while the total serum cholesterol was increased 30%. The reduction in adrenal ascorbic acid and cholesterol and the corresponding increase in blood ascorbic acid and serum cholesterol indicate the significance of observed changes in adrenal size during dietary restriction and rehabilitation.

Occurrence of dermatitis in rats fed diets containing lactose as the sole carbohydrate. R. A. COLLINS,* M. SCHREIBER* AND C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

During studies on the nutritive value of goat's milk, cow's milk and purified lactose diets a scaly dermatitis was observed upon the fore and hind paws of the white rat. The maximum degree of dermatitis appeared when weanling rats were fed the milk or the lactose diets for 2 weeks. After this time the dermatitis spontaneously improved. Diets of goat's milk and cow's milk produced the scaly paw condition in the fall, winter and spring, but during the summer these milk diets failed to produce a severe dermatitis. Purified lactose diets containing 28% corn oil or 28% cocoanut oil produced a more severe dermatitis than did diets containing butterfat or lard. A study using various carbohydrates in the 28% corn oil diet demonstrated that only lactose produced the characteristic dermatitis and that sucrose, dextrin and a glucose-galactose mixture were without effect. The supplementation of 1 to 2 grams of fresh beef liver per rat per day both prevented and rapidly cured the dermatitis. The withdrawal of the active liver supplement from milk diets allowed the dermatitis to appear within 5 days, thus the anti-dermatitis principle of beef liver is not stored by the rat to any appreciable extent. Supplementation of all known water soluble and fat soluble vitamins, ethyl linoleate, purified liver extracts, streptomycin, glutathione or amino acids had no definite curative activity.

Effect of penicillin on growth, egg production and hatchability. J. R. COUCH, J. F. ELAM* AND L. L. GEE.* *Texas Agricultural Experiment Station, College Station, Texas.*

The first group of 21 depleted crossbred chicks (N.H. × S.C.W.) was fed a basal ration composed of 60% ground yellow corn, 35% soybean oil meal, 2% bone meal and 1½% oyster shell plus manganese sulphate, riboflavin, calcium pantothenate, and an A and D supplement. The 2nd group was injected with B_{12} (2 μg/chick/week), the 3rd fed penicillin (33 mg/kg.) and the 4th group was injected with B_{12} and fed penicillin. Weekly bacterial counts were made on fecal samples by standard bacteriological techniques. Females from the unsupplemented group were given weekly intravenous injections of penicillin (2 μg.) after the 14th week. The weight of the birds was increased by feeding penicillin. Females fed penicillin and injected with B_{12} were heaviest at 20 weeks of age. Injection of penicillin intravenously produced females that weighed about the same as those given B_{12} at 20 weeks. The percentage egg production for *groups I, II, III* and *IV* for the first 2½ months of the laying period was 29, 38, 22 and 50 respectively. Hatchability of eggs collected from pullets injected with penicillin and from *groups II, III* and *IV* was 0%, 71%, 44% and 79% respectively. Fecal bacterial counts revealed that the feeding of penicillin increased the total count, the Enterococci, the yeasts and the penicillin resistant bacteria. The intravenous injection of penicillin produced a change in the microflora similar to that described above.

Evidence of two unidentified factors required by the chick. W. W. CRAVENS, H. W. BRUINS,* M. L. SUNDE* AND E. E. SNELL. *Depts. of Poultry Husbandry and Biochemistry, Univ. of Wisconsin, Madison.*

A preliminary report (*Poultry Sci.* 29: 627, 1950) showed that a purified diet containing an isolated soybean protein would not support chick growth and livability though the diet was complete in the known dietary essentials. Some supplements, notably wheat bran, gave fair results. Further studies have given clear evidence that at least two factors are involved. If 10 or 20% bran is added good livability will obtain but growth will not exceed 180 grams at 3 weeks. However, if 10% Torula yeast is fed in addition to 10% bran, growth of 225 grams is observed, while feeding yeast alone results in high or complete mortality. This indicates the presence of two factors since yeast alone (10%) is not equivalent to 10% bran but when combined with bran it stimulates growth more than an additional 10% of bran. Chick assays for the 'bran factor' and the 'Torula yeast factor' have been designed. The bran factor is found in many plant materials. Other good sources of the yeast factor have not been found. Animal products tested are not good sources of either factor. A deficiency of either factor results in high mortality at about 2 weeks. No specific deficiency syndromes have been observed. A severe loss of weight usually occurs before death. Stability of the factors has been studied and are reported. Data relating to the effect of carbohydrate and antibiotics are also discussed.

Serum cholinesterase of male albino rat during food restriction and subsequent protein rehabilitation. ESTHER DA COSTA AND RUTH CLAYTON (introduced by CARL J. KOEHN). *U. S. Army, Med. Nutrition Lab., Chicago, Ill.*

Serum cholinesterase activity was measured in 182 male albino rats after 5, 10 or 20 weeks on a

control, high protein (60%), low calorie or modified carrot (2% protein) diet by a decrease in pH per hour (ΔpH/hr.). The average ΔpH/hr. for rats on the control, high protein, low calorie or the modified carrot diet was 0.235, 0.259, 0.254 and and 0.191, respectively. After 5 and 10 weeks on either the low calorie or modified carrot diet rats were rehabilitated on either a high protein diet *ad libitum* or with B_{12} (2.5 μg/rat) or 11 gm/rat/day. Serum cholinesterase was determined after ¾, 2, 3, 7, 35 and 70 days of rehabilitation. After 18 hours of rehabilitation with the high protein diet *ad libitum* following 5 weeks on the low calorie diet a Δ pH/hr. of 0.375 was found and after 10 weeks, 0.325. After 1 week of rehabilitation with a high protein diet *ad libitum* and with B_{12} following 10 weeks on the modified carrot diet an average Δ pH/hr. of 0.328 and 0.334 respectively was found, otherwise the trend was toward the control level. Since changes in serum cholinesterase activity and body weight usually parallel each other, this test may serve as an estimate of the nutritive state of an animal.

Effect of vitamin C on pantothenic acid-deficient rats. Floyd S. Daft. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

It is well known that weanling rats maintained on a purified diet deficient in pantothenic acid show growth failure and develop such deficiency signs as achromotrichia and porphyrin-staining of the fur and whiskers. They succumb, often within a few weeks, and usually with some degree of adrenal pathology. If, however, the pantothenic acid-deficient diet given the weanling animals contains vitamin C at a level of 2%, the development of the deficiency syndrome in most animals (in tests to date) is at the least greatly delayed or modified. The effect on growth is striking. There appears also to be an effect on porphyrin accumulation on the fur and whiskers. The average length of life of the animals is greatly increased. Histological examination of the adrenals has not as yet been made and studies of achromotrichia are still incomplete.

Uptake of radioactive vitamin B_{12} by bacteria in single and mixed cultures. R. L. Davis,* L. L. Layton* and Bacon F. Chow. *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

In view of the lack of agreement as to the role of the intestinal flora with respect to various nutrients in the gut, an *in vitro* study of vitamin B_{12} metabolism of certain bacteria is being made. Culture media containing vitamin B_{12} labeled with Co^{60} were inoculated with various types of bacteria. After incubation, the resultant bacterial cultures were centrifuged; the washed cells and media were assayed for radioactive Co^{60} as $CoSO_4$ and CoS respectively, using a Geiger-Müller counter. Aliquot samples of media (80 ml.) containing 4 mμ vitamin B_{12} with total activity of 60 cpm were inoculated with *L. leishmannii*, *E. coli*, *S. lactis R* and *L. arabinosus*. After 45 hours' incubation, the cells were separated, washed and assayed for radioactivity. It was found that about one-half of the added activity was present in the first 3 organisms mentioned above and a negligible amount in the last one. Competitive systems of bacteria were also studied; the types of organisms cultured were separated by cellophane membranes through which vitamin B_{12} was diffusible. In one such system in which *L. leishmannii* and *L. arabinosus* were separated by cellophane and 6 mμ labeled vitamin B_{12} included in the media, subsequent assay for radioactivity of the grown cells and media demonstrated that *L. leishmannii* absorbed the same proportion of this activity as when cultured alone. Data on the behavior of other competitive bacterial systems for radioactive vitamin B_{12} are also presented as well as methods for the isolation of Co^{60}.

Retention of nitrogen by 13-year-old girls on two levels of intake. Gladys Derby,* Doretta Schlaphoff* and Frances A. Johnston. *N. Y. State College of Home Economics, Cornell Univ., Ithaca.*

Six girls, 13 years of age, were fed a controlled diet for 9 weeks. The first week was an adjustment period. During the first experimental period of 4 weeks the mean daily intake was 76.3 gm. of protein or 8.245 gm. of nitrogen per square meter of surface area and the daily mean energy content of the diet was 2690 calories. On this intake the mean daily amount of nitrogen retained was 1.452 gm. or 0.981 gm. of nitrogen per square meter. During the second 4-week period the intake was raised to 87.4 gm. of protein or 9.318 gm. of nitrogen per square meter and 2880 calories. On this diet the mean daily amount of nitrogen retained was 2.174 gm. or 1.449 gm. of nitrogen per square meter.

Acute vitamin E deficiency in the monkey. James S. Dinning,* Lloyd D. Seager and Paul L. Day. *Dept. of Biochemistry and Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock.*

The syndrome of acute vitamin E deficiency in monkeys is characterized by creatinuria, elevated allantoin excretion, muscular dystrophy and leucocytosis. Allantoin excretion was elevated more than 6-fold during the deficiency state. Urinary creatine reached values as high as 120 mg. daily/kg. body weight. The leucocytosis was due primarily to elevation in number of granulocytes. All these symptoms responded to therapy with synthetic alpha tocopherol acetate. Electrocardio-

graphic tracings indicated cardiac involvement in a monkey showing irreversible deficiency but no appreciable changes in a monkey in which the signs of deficiency were reversible.

Effect of storage on tissue tocopherols. MEI YU DJU,* LLOYD J. FILER, JR.* AND KARL E. MASON. *Dept. of Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Autoxidation of fats and development of off-flavors are known to occur in tissues and foodstuffs subjected to prolonged storage at low temperature. Furthermore, in the quantitative analysis of tissue tocopherol it is necessary to know what losses of tocopherol are incurred during storage of the sample. Using the method of 'dry' homogenization described by Quaife and Dju (*J. Biol. Chem.* 180: 263, 1949), samples of human liver and adipose tissue were prepared and analyzed in duplicate immediately and periodically during 20 weeks of storage at −20°C. The extracted fat was characterized as to iodine value and peroxide value before and after molecular distillation. Ten weeks of storage produced no change in the tocopherol content of liver but after 20 weeks the determined tocopherol was 87.5% of the original concentration (0.128 mg/gm. fat). The tocopherol content of adipose tissue was significantly lowered by the 4th week of storage (78.0%) and by the 10th and 20th weeks of storage was 61.2% and 53.7% of the original concentration (0.054 mg/gm. fat). Mean iodine values of the liver and adipose tissue fat were 94.7 and 69.3, respectively, with storage and molecular distillation producing no significant change. It is apparent that tissue which is rich in fat and tocopherol, but probably deficient in other biological antioxidants, shows an early decrease in tocopherol content during low-temperature storage. (Supported by a grant from the National Vitamin Foundation, Inc.)

Utilization of *d*-biotinol by the rat and human. L. DREKTER,* J. SCHEINER,* E. DE RITTER* AND S. H. RUBIN. *Nutrition Labs., Hoffmann-La Roche Inc., Nutley, N. J.*

D-biotinol, the alcohol analog of *d*-biotin, is as effective as the latter in curing rats of a biotin deficiency induced by feeding egg white. The biotinol is converted to biotin and excreted as such in the urine. After oral administration to rats at physiological levels of 10 to 50 μg., the urinary excretion is equivalent to about one-third of the dose of either biotin or biotinol. Similarly, after intramuscular injection of comparable amounts, the percentage excretion in the rat urine represents about 40% of the dose of both compounds. Increasing the oral dose to therapeutic levels of 3.4 to 10 mg. results in a significantly greater urinary output of biotin after biotinol than after biotin. Most of this difference—which is not observed after intramuscular dosage—may be accounted for on the basis of greatly increased fecal excretion of biotin after dosage of biotin as compared to biotinol. Urinary excretion tests in a 33-month-old child indicate that biotinol is converted to biotin in the human after either oral or intramuscular administration. The limited nature of the data and the fluctuations in excretion levels in the human preclude the establishment of a precise index of relative utilization of biotin and biotinol, but the excretion levels are similar for both compounds. Biotinol is inactive for *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Saccharomyces cerevisiae*, the microorganisms commonly used for biotin assay.

Possible relation between flavonoids and adrenal cortex activity. WALTER H. EDDY AND BORIS SOKOLOFF.* *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

There are no data on the distribution of flavonoids in mammalian tissue. It therefore seemed desirable to investigate the distribution of flavonoids in the adrenal gland and spleen and their possible relation to the activity of the adrenal gland. Our investigation shows that the adrenal gland contains significant amounts of at least two flavonoids, one with absorption peak at 450 mμ and the other at 400 mμ (borocitrate reaction). The spleen contains at least one of these compounds. Administration of a flavonoid compound to adrenalectomized rats prolonged the life cycle. In this series, British breed of rats of 151-gram average weight were adrenalectomized. At the end of 2 weeks 22 rats developed symptoms of adrenal insufficiency. One group of 12 rats was given 5 mg/100-gm. wt. daily of a flavonoid compound. This group survived 56 days (average) against the control rats (10) which survived 28 days (average). In the next series of experiments, August rat carcinoma 175-G was used. Two extra adrenals were grafted to each of 12 rats two days before the transplantation of carcinoma 175-G. These rats were given injections of 10 mg/100 gm. wt. of flavonoid compound daily for 15 days. There was significant inhibition of tumor growth. The average size of tumor at the end of three weeks was 2.5 cm^3 as against 8.6 cm^3 in the control group with two extra adrenal glands but not receiving the flavonoid compound. The graft of adrenal gland by itself produced no apparent effect on tumor growth. The flavonoid compound by itself inhibited but slightly the growth.

Neopyrithiamine—a thiamine antagonist. GLADYS A. EMERSON AND DOROTHEA CASEY.* *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Wilson and Harris reported the synthesis and chemical properties of neopyrithiamine (*J. Am.*

Chem. Soc. 71: 2231, 1949), a compound which has the structure originally assigned to pyrithiamine. Eusebi and Cerecedo (*Science* 110: 162, 1949) found that neopyrithiamine was a more effective antagonist of thiamine for the mouse than was oxythiamine. The index of inhibition (inhibitor/vitamin) was not established. Neopyrithiamine has been found to be approximately 8 times more effective as an antagonist for thiamine than pyrithiamine which was reported by Woolley (*J. Biol. Chem.* 149: 285, 1943) to have an index of inhibition of 40:1. A low intake of neopyrithiamine (5γ/day) hastened the onset of thiamine deficiency in rats conditioned by partial depletion of their stores of thiamine. Concomitant administration of 5γ thiamine daily did not completely reverse this antithiamine effect. Complete neutralization occurred at this level of thiamine intake when the index of inhibition was of the order of 5:1. This ratio was only slightly changed by increasing the thiamine intake stepwise to 50γ daily. The index of inhibition in animals receiving from weaning very high intakes of thiamine (7.5γ/gm. of ration), either admixed with a purified diet or as contained in a natural food ration, was approximately 25:1.

Nutritional edema in rats fed diets low in choline and protein. R. W. Engel and H. D. Alexander.* *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn, Ala.*

Diets were devised that produced severe nutritional edema, characterized by hydrothorax, ascites and extensive subcutaneous fluid deposits over the body surface in rats. This dietary deficiency was produced by gradually reducing the dietary protein constituents, water extracted casein and alcohol-extracted peanut meal. The protein concentration was reduced 10% every 2 weeks, beginning with the 3rd, 5th, 7th and 9th weeks until the protein level of the diet had been reduced from an original 18% to a final 7%. The dietary choline concentration, initially 0.1%, was gradually reduced to zero by the end of the 4th week. The nutritional edema was also produced by feeding diets containing only 7% protein and no choline from weaning. Under these conditions the body weights remained nearly constant and only an occasional animal died with acute choline-deficiency kidney hemorrhage. When the latter diets were used weanling rats developed severe edema in from 60 to 120 days whereas when the high-protein diet was fed initially 150 to 200 days were required to produce edema. The edema was accompanied by low blood hemoglobin concentrations, ranging from 3.0 to 7.0 gm/100 ml. of blood. Abnormal liver conditions were also noted ranging from severe fatty infiltration to varying stages of fibrosis and nodular cirrhosis. Supplementing these diets with choline at a level of 2 gm/kg. of diet prevented the development of edema, anemia and liver damage.

Fat deposition in liver and carcass of the mouse. Paul F. Fenton and Jeanne S. Mershon.* *Dept. of Biology, Brown Univ., Providence, R. I.*

The effect of varying the fat content of the diet on the rates of weight gain of 4 highly inbred strains of mice was investigated. The purified diets employed were complete (as far as our knowledge of the nutritional requirements of the mouse permits) and contained 5, 15, 25 or 47.5% fat. Mice of the A and C3H strains showed greater weight gains when fed the higher fat diets. Much of this increase in weight gain was due to greater fat deposition. As much as 38% total body fat has been found by analysis. On the other hand mice of the C57 and I strains showed the same weight gains regardless of the dietary fat content. It has been noted in several laboratories that mice carrying the yellow gene will, after continued inbreeding, no longer show their original tendency to obesity. Feeding a high fat, purified diet resulted in significantly greater weight gains of yellow mice as compared with their non-yellow litter mates. It has been noted in previous studies that mice maintained for several months on presumably complete purified diets developed fatty livers. With the use of A and C3H strain mice fed suitable rations it has been possible to induce fatty liver in every case examined. Addition of liver powder to the diet considerably reduced the liver fat. Vitamin B12 was ineffective. Fatty livers were not seen in mice of these strains when raised on a commercial feed.

Function of nicotinic acid in protein metabolism. G. Fraenkel. *Dept. of Entomology, Univ. of Illinois, Urbana.*

The nicotinic acid requirements of two insect species, *Tribolium confusum* and *Tenebrio molitor*, were studied in relation to the protein content of their diets. On normal diets, containing 20% protein (casein), 8 to 16 μg. nicotinic acid are required per gram of the dry diet for optimal growth. No growth takes place in the absence of nicotinic acid and no amount of additional tryptophane can compensate for its lack, showing that the level of protein in the diet can have no effect on the supply of nicotinic acid. This phenomenon, unique among multicellular animals, makes it possible to study nicotinic acid requirements in relation to the protein content of the diet. It was found that the nicotinic acid requirements were roughly doubled with a doubling of the amount of casein present. For *Tribolium*, the minimum dose for optimal growth was 4 μg/gm. of the dry diet with 5% casein, and 64 μg/gm. with 75% casein. For *Tene-*

brio corresponding figures were 8 μg/gm. with 5% casein, and 128 μg/gm. with 50% casein. It has been shown that the large requirements at high casein levels are due rather to the large proportion of protein than the small proportions of carbohydrates. The suggestion is offered that nicotinic acid plays a part in the utilization of protein, possibly for energy requirements.

Comparison of distribution of carotenoid component in tissues of several species. J. GANGULY* AND H. J. DEUEL, JR. *Dept. of Biochemistry and Nutrition, Univ. of Southern California, Los Angeles.*

The presence of small amounts of β-carotene was demonstrated in the blood, liver, ovary and fat of chickens previously on diets containing β-carotene or after oral administration of this carotenoid in spite of the fact that the chicken changes carotene to vitamin A in the intestinal wall (Cheng *et al. J. Nutrition* 41: 619, 1950). Traces of α-carotene occur after oral feeding while cryptoxanthin and zeaxanthin appear in considerable amounts in the blood and liver after their administration. Although lutein is the predominant carotenoid in all the tissues of hens on normal diets, it was possible to raise chickens on synthetic carotenoid-free diets to maturity. Such birds produced eggs which were practically carotenoid-free as were their tissues. In the case of the ewe, traces of both β-carotene and lutein occur in the liver but not in the corpora lutea, while in the cow extremely high levels of β-carotene were found in blood, liver and corpora lutea along with small amounts of lutein. Chickens selectively absorb carotenols which pass into the egg while the cow stores chiefly carotenoids in all tissues with traces of lutein. The identity of all compounds was established chromatographically and spectrophotometrically. (Aided by a grant from the Nutrition Foundation.)

Influence of carbohydrate on in vivo fat metabolism. R. P. GEYER AND W. R. WADDELL.* *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Palmitic acid oxidation by the rat was decreased by the prior oral intubation of glucose or sodium pyruvate. Carboxyl-labeled palmitic acid or tripalmitin was administered intravenously in the form of a highly dispersed emulsion in which olive oil was used as the carrier, and phosphatide and monostearin were used as stabilizers. Approximately 50 and 70% of the free acid and triglyceride were oxidized ($C^{14}O_2$), respectively in 24 hours. Prior carbohydrate supplementation decreased the rate of oxidation about one-half, whereas fasting increased the rate of oxidation of both lipids. Changes in the rate of oxidation were reflected in corresponding changes in the specific activity of the CO_2 and un-oxidized substrate was incorporated into tissue neutral fat and phospholipids. Octanoic and lauric acids were rapidly and fairly completely oxidized by the normal rat and were little affected by carbohydrate or fasting.

Niacin depletion and requirement in man. GRACE A. GOLDSMITH, HERBERT P. SARETT, U. D. REGISTER* AND JANIS GIBBENS.* *Nutrition Research Lab., Tulane Univ. School of Medicine, New Orleans, La.*

Wheat or corn diets containing approximately 250 and 200 mg. of tryptophan and 6 and 5 mg. of niacin per day, respectively, were given to 4 adult females. *Subject 1* received the wheat diet for 3 months and *subjects 2* and *3* the corn diet for 6 weeks without showing signs suggesting nutritional deficiency. Later, *subject 2* received the corn diet for 3 months and the following findings developed, in sequence, after the 7th week: pyrosis, changes in the lingual papillae, white patches under the tongue, skin infection of the vulva, apathy, sore mouth, anorexia, dysphagia, cheilosis, angular stomatitis, crusting of the elbows, erythema of the mucous membranes, depression, reduction in hemoglobin and bloody diarrhea. Following niacinamide administration, 10 mg. three times daily, most of the lesions healed within 1 week, those of the tongue and elbows within 1 month. *Subject 4* has been receiving the corn diet for 4 months and has shown minor tongue changes, mild intermittent cheilosis, amenorrhea and thickened, pigmented skin of the extensor surface of the fingers. All subjects remained in nitrogen balance, maintained body weight and showed no change in basal metabolic rate, glucose tolerance or serum protein concentration. After a brief period, the urinary excretion of N^1-methylnicotinamide decreased to 1 mg. or less per day and no 6-pyridone of N^1-methylnicotinamide was detectable. The findings suggest that the 5 mg. of niacin in the corn diet was slightly below the minimal requirement of these subjects.

Iron absorption by women: comparison of three ferrous salts. MARY R. GRAM* AND RUTH M. LEVERTON. *Human Nutrition Lab., Agricultural Experiment Station, Lincoln, Nebr.*

The subjects for this study were 83 normal women ranging in age from 18 to 42 years and living on self-chosen diets. Twenty-seven women were given ferrous gluconate, 29 ferrous lactate, and 27 ferrous sulfate daily for 4 weeks. The dosage of each salt was calculated to supply approximately 100 mg. of iron/day. The dietary iron was estimated at 10 mg/day. Hemoglobin was determined on the 2 consecutive days before medication began, weekly during the 4 weeks of medication, then weekly for 4 weeks after medication ceased.

The trend of the mean hemoglobin values expressed in grams % for each group follows:

	GLUCONATE	LACTATE	SULFATE
Initial	13.4	13.2	13.1
After 2 weeks with medication	13.3	13.2	13.2
After 4 weeks with medication	13.2	13.3	13.3
Four weeks after medication	13.4	13.3	13.4

None of the ferrous salts appreciably affected the hemoglobin values. Complete fecal collections were made during the 3rd and 4th weeks of medication. Feces were analyzed for iron using the reagent, ortho-phenanthroline. Mean daily iron retention for subjects given ferrous gluconate was 8.9 mg., for subjects given ferrous lactate 12.5 mg., and for subjects given ferrous sulfate 10.6 mg. Analysis of variance indicates no statistically significant difference among these means.

Effect of 5, 6-dimethylbenzimidazole and related compounds on the growth of *L. lactis* Dorner. DAVID HENDLIN AND MARGARET H. SOARS (introduced by GLADYS A. EMERSON). *Merck & Co., Inc., Rahway, N. J.*

5,6-Dimethylbenzimidazole has been reported as a degradation product obtained from vitamin B_{12} on acid hydrolysis. The ability of 5,6-dimethylbenzimidazole and some related compounds to replace vitamin B_{12} in the nutrition of the rat has been described. None of these compounds possessed vitamin B_{12} activity for *Lactobacillus lactis* Dorner when tested at levels as high as 1 mg/ml. More than that, the benzimidazole compounds were found to inhibit markedly the growth of *L. lactis* in the presence of as much as 1 μg/ml. of vitamin B_{12}. The following compounds possessed inhibitory properties: 1,2-diamino, 4,5-dimethylbenzene, 5-methyl-, 4,5-dimethyl and 5,6-dimethylbenzimidazole. No inhibition of growth was obtained with benzimidazole, 1-methyl-,2-methyl-, 4-methyl-, and 2,5-dimethylbenzimidazole at levels as high as 1 mg/ml. The inhibition obtained with 1,2-diamino, 4-5-dimethylbenzene was competitively antagonized by vitamin B_{12} over a narrow range of inhibitor. This was not the case with 5,6-dimethylbenzimidazole. Higher levels of the substituted diaminobenzene produced inhibition which could not be reversed by the known B vitamins, purines, pyrimidines, nucleosides and nucleotides, singly or in combination.

Large-scale preparation of coenzyme A concentrates. HARVEY HIGGINS,* R. E. HANDSCHUMACHER,* D. A. BUYSKE,* L. J. TEPLY*, G. C. MUELLER*, V. H. CHELDELIN, T. E. KING* AND F. M. STRONG.* *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

A convenient procedure has been developed for obtaining large quantities of stable coenzyme A concentrates from hog liver. One hundred pounds of trimmed livers taken directly from the slaughter floor are quickly chilled, coarsely ground, and dropped into an equal weight of boiling water. Live steam is passed through the mixture until the temperature has been maintained at 85–95° for 5 minutes. The mixture is then chilled with 180 pounds of crushed ice, filtered, and the clear filtrate adsorbed on 500 gm. of 40–100 mesh Nuchar C-190. Elution is best accomplished with aqueous alkali at pH 9–10. The use of pyridine or acetone appears to offer no advantage. Concentration of the neutralized eluate to one-half its volume, followed by adjustment to pH 2 and addition of 4 volumes of acetone precipitates the active fraction as a pale buff or yellow powder. The procedure requires about 3 working days. The success of the method depends on preventing autolysis and using a suitable charcoal. The product contains about 1–2% of bound pantothenic acid, corresponding to 4–8% of coenzyme A, and is obtained in a yield of 10–12 gm. Earlier samples eluted with aqueous pyridine were essentially free of cocarboxylase but contained considerable amounts of DPN, TPN, and probably riboflavin coenzymes.

Relation of dietary glycine and alanine to calcium metabolism. JAMES F. HOGG (introduced by J. P. CHANDLER). *Dept. of Biological Chemistry, University of Michigan, Ann Arbor.*

Previous work with certain bacteria has shown that glycine and alanine cause a growth inhibition which is fully reversible by calcium salts. Sherman and Ragan have reported that increased dietary protein will, on a diet previously just sufficient in calcium, deplete the body calcium reserves. Investigation was made to learn if glycine and alanine are the cause of this effect of protein on calcium retention. White rats were fed low calcium diets which contained: *a*) 20% casein, *b*) 30% casein, *c*) 20% casein and 10% gelatin and *d*) a combination of casein, glycine and alanine which equalled *c* in content of nitrogen, glycine and alanine. The experiment was terminated when each animal had consumed 1 kg. of diet (within 60–65 days). Urine and feces samples, collected between the 2nd and 7th days, and the entire animal bodies were analyzed for their calcium content. *Groups b* and *c* had equal body calcium levels whereas *group a* was definitely lower and *group d* was definitely higher in total calcium. Thus only total protein level and free glycine and alanine affected the total calcium retention. As to calcium excretion *group a* had a distinctly lower fecal excretion and *group d* was markedly higher in urinary excretion. It has not been possible to show a calcium-depleting effect of dietary protein but rather the opposite effect.

Creatine formation in CCl_4 poisoned rats as influenced by dietary α-tocopherol, vitamin B_{12} and cystine. E. L. HOVE AND J. O. HARDIN*. *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.*

Rats reared on synthetic type diets with 10% casein were subjected to weekly sub-lethal CCl_4 injections. Supplements of L-cystine, DL-α-tocopherol acetate and vitamin B_{12} in all combinations were added, or the casein was increased to 18% with and without tocopherol. The creatinurea associated with the vitamin E deficiency was doubled by the CCl_4 injections among rats on the 10% casein diet but was not influenced in rats on the 18% casein diet, or in rats on the 10% basal diet supplemented with cystine. Vitamin B_{12} increased the creatinurea except in the presence of both cystine and vitamin E. Determinations were made on the ability of liver slices from rats on the various dietaries to synthesize creatine, *in vitro*, from guanidoacetic acid and methionine. Conditions resulting in high creatinurea such as vitamin E deficiency or CCl_4 toxicity were associated with low creatine synthesis by liver slices, except when vitamin B_{12} was included in the diet. In these cases not only was the synthetic activity of the liver increased, but methionine was not necessary in the substrate. The conclusion drawn from the results on growth, growth deficit due to CCl_4, creatinurea, tooth color and other criteria is that the 10% casein diet is made equal to the 18% casein diet by the addition of *both* cystine and vitamin B_{12}. In the absence of these factors the vitamin E requirement of the rat is increased and vitamin E is involved in the synthesis of creatine from guanidoacetic acid and labile methyl.

Effect of pantothenic acid deficiency on skin copper. JAMES M. HUNDLEY AND ROBERT B. ING. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

Weanling, Long-Evans black rats fed a purified diet adequate in vitamins, except for pantothenic acid, developed marked growth restriction and gray hair. Skin copper analyses were done in these rats, in littermate rats receiving adequate pantothenic acid, and in similar rats on stock diet. Eight skin specimens from 4 normal rats subsisting on Purina Dog Chow (21.5 μg. of Cu/gm.) showed 31.4 μg. of Cu/gm. (all figures based on dry weight). Eight specimens from 4 rats on the pantothenic acid supplemented purified diet (14.6 μg. of Cu/gm.) showed 38.3 μg. of Cu/gm. In striking contrast, 10 skin specimens from 5 gray-haired pantothenic acid-deficient rats showed 162.5 μg. of Cu/gm. In a subsequent experiment 19 weanling rats were fed the pantothenic acid-deficient diet until gray hair was well developed (7 weeks). They were then divided into 2 groups. One group (10 rats) was sacrificed and 208 μg. of Cu/gm. of skin was found. The second group (9 rats) was treated with pantothenic acid for 2 weeks. Marked gain in body weight and beginning regeneration of black hair were noted. Analysis of 15 skin specimens from these rats showed 39.7 μg. of Cu/gm. These findings suggest that pantothenic acid deficiency may produce gray hair by blocking the utilization of Cu in hair growth and melanin formation.

Metabolism of deuteriumated lauric acid. MERA R. JETTON*, HENRY SHERMAN* AND ROBERT S. HARRIS. *Nutritional Biochemistry Labs., Massachusetts Inst. of Technology, Cambridge.*

Deuteriumated lauric acid (5.02 atom %) was prepared by an exchange reaction with heavy water, platinum oxide being used as a catalyst, and then converted into trilaurin. Trilaurin and olive oil were randomly rearranged by treatment of the mixture (1:2) with sodium methoxide at 60°C. for one hour. The resulting fat was fed *ad libitum* to adult male rats at a level of 14% in a nutritionally adequate synthetic diet. Groups of animals were sacrificed after 0, 7, 14, 28 and 56 days on the diet, and the fatty acids extracted by saponification of the lungs, spleens, testes, kidneys, brains, hearts, livers, carcasses and hides. In addition, samples of water were obtained from these tissues, as well as from the blood and urine. Glycogen was extracted from the livers and carcasses. All samples were analyzed for deuterium by the 'falling-drop' technique. Detectable amounts of deuterium appeared in the urine within 24 hours after the ingestion of the tagged fat, indicating that lauric acid is rapidly metabolized. The deuterium concentration of the water of all tissues increased slightly with time and was identical for all the tissues analyzed. The deuterium content of all tissue fatty acids also increased with time, the brain fatty acids showing the lowest concentration and the heart fatty acids the highest. The deuterium content of the liver fatty acids was lower than that of the carcass fatty acids, a finding which is the reverse of our results with isooleic acids and which indicates a difference in the metabolism of lauric and oleic acids.

Folic acid metabolism in normal male subjects. DORIS JOHNSON* AND HELEN T. PARSONS. *Dept. of Home Economics, Univ. of Wisconsin, Madison.*

The urinary excretion levels of 10 university men consuming qualitatively restricted diets and supplements of 1, 2 and 5 mg. of pteroylglutamic acid were determined microbiologically. On the basal diet an average of 3.0 mg. (range 0.9–4.3) of folic acid per day was excreted in the urine, an average of 0.75% of the calculated intake. Five of these subjects ingesting a 1-mg. test dose of folacin excreted an average of 5.5% (range 3.3–

8.3) of the test dose in the urine; 5 ingesting 2 mg. excreted an average of 14.5% (range 5.2–20.2); 3 ingesting 5 mg. excreted an average of 39.2% (range 37.5–40.5). These urinary values for the Wisconsin men on a 1-mg. dose are only 2.8% higher than the average for the 15 Wisconsin young women reported last year, but 16.5% below the average for 4 men published from another laboratory. On a 2-mg. dose the Wisconsin men were 28.5% below these other men; at 5 mg. the difference is 10% while between the Wisconsin men and women the difference is 1%. Determinations of the citrovorum factor have been made. Vitamin C which has been suggested to influence folacin metabolism was adequately included in the diet.

Vitamin B_{12}, methionine, choline and related substances as growth factors for chicks. THOMAS H. JUKES AND E. L. ROBERT STOKSTAD. *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Various commercial soybean fractions were used as sources of protein in simplified diets which were fed to vitamin B_{12}-deficient chicks. *Diet 1* had the composition glucose (Cerelose) 62.5 gm.; soybean protein, 25 gm.; calcium gluconate, 50 gm.; corn oil *plus* vitamins A, D and E, 3 gm.; bone ash, 2 gm.; salt mixture, 2 gm.; cystine, 0.3 gm.; dimethylaminoethanol HCl, 0.2 gm.; inositol, 0.1 gm.; aureomycin HCl, 2 mg.; thiamine, 1 mg.; riboflavin, 1 mg.; niacinamide, 5 mg.; pyridoxine, 1 mg.; calcium pantothenate, 5 mg.; vitamin K compound, 0.5 mg.; biotin, 0.02 mg. The growth response to homocystine in chicks on this basal diet was not increased by folic acid but was increased by vitamin B_{12}, betaine or choline. The response to homocystine *plus* choline was increased by either folic acid or vitamin B_{12}. The response to homocystine plus betaine was less than the response to homocystine plus choline in the presence of either folic acid, vitamin B_{12} or vitamin B_{12} plus folic acid. The average growth response to methionine was not increased by the further addition of choline; indicating that the dimethylaminoethanol content of the basal diet supplied the essential portion of the choline molecule and that the growth response to choline in the presence of homocystine was due to the function of choline in methylating homocystine. Experiments with other diets indicated that the response to aureomycin was affected by the composition of the diet.

Growth-promoting activities of antibiotics and yeast cultures for chicks and turkey poults. F. H. KRATZER, C. R. GRAU, MORTIMER P. STARR* AND D. M. REYNOLDS*. *Divs. of Poultry Husbandry and Bacteriology, College of Agriculture, Univ. of California, Davis and Berkeley.*

The growth response observed when streptomycin and other antibiotics are fed to turkey poults was studied in relation to the numbers of yeast organisms present in the intestinal tract. Poults fed streptomycin-containing diets exhibited a 5- to 10-fold increase in numbers of intestinal yeast organisms over control poults. Cultures of a yeast isolated from intestinal contents were fed as supplements (1% of the diet) to adequate poult and chick diets and the growth rates obtained were compared with those of birds fed normal diets or supplements of antibiotics. One strain of yeasts isolated from the intestinal tract of streptomycin-fed poults produced a slightly more rapid growth than normal diets in some, but not in all, chick and poult feeding trials. This slight growth response is probably different from that obtained from streptomycin feeding. Day-old chicks given access for the first 3 days of life to droppings of normal or aureomycin-fed month-old chicks grew slower than control chicks. Autoclaved droppings produced no growth depression. Droppings from birds fed aureomycin produced less growth depression than droppings from normal birds. Aureomycin improved growth of the chicks fed either fresh or autoclaved droppings.

Biological activity of 6,7-diethyl-9-(D-1^1-ribityl) isoalloxazine (diethyl-riboflavin). J. P. LAMBOOY (introduced by E. S. NASSET). *Univ. of Rochester, Dept. of Physiology and Vital Economics, Rochester, N. Y.*

As previously reported, diethyl-riboflavin has activity which is almost equivalent to that of riboflavin for *Lactobacillus casei* 7469, throughout the range of limiting concentrations. It can now be reported that the analog also has activity for *Bacillus lactis acidi*, another strain of bacteria requiring riboflavin. In spite of the ability of diethyl-riboflavin to support growth of these micro-organisms no activity has been observed for white rats throughout ranges of 3.2 to 129 μg/rat/day or of 8.1 to 32.3 μg/gm. of food.

Effect of the time factor and calorie level on nitrogen utilization of young women. RUTH M. LEVERTON, MARY R. GRAM* AND MARILYN CHALOUPKA.* *Human Nutrition Lab., Agricultural Experiment Station, Lincoln, Nebr.*

Sixteen young women served as subjects for this study. One group of 8 subjects had a daily protein intake of 43 gm., and the other group of 8 had 63 gm., otherwise the dietary control was identical for the two groups. The 54 days of the study were divided into 3 consecutive 18-day periods with calorie intakes of 1800, 2400, and 1800 respectively. The last 9 days of each period was a metabolism period. In the first and second periods there was no animal protein in the noon meal but in the third period the total milk intake was redistributed so that animal protein in the form of milk

was present in the noon meal as well as in the morning and evening meals. For both groups, increasing the caloric intake from 1800 to 2400 Cal. had a definite sparing action on nitrogen. The group on the lower protein intake had a significantly lower urinary excretion in the third period when there was animal protein in every meal than in the first period when it was absent in the noon meal. The other group showed no difference in urinary excretion between the first and third periods. It is concluded that omitting animal protein from the noon meal adversely affects nitrogen metabolism when the daily protein intake is as low as 43 gm., but does not affect it when the intake is 63 gm.

Supplemental value of cystine and methionine for low protein (casein) diets. HOWARD B. LEWIS AND RUTH S. FAJANS.* *Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.*

The comparative supplemental values of cystine, methionine, alone and in combination in varying proportions, for a low casein diet (7.5%), as evidenced by promotion of growth, was studied in young male white rats. Since it was desired to rule out, so far as possible, the requirement of methionine as a source of methyl groups, adequate amounts of choline chloride and liver extract were present in the diets. The relative protein efficiencies (increment of weight per gm. of protein consumed) over a period of 28 days were determined, and the results were analyzed statistically. The basal casein diet which supplied 27 mg. cystine and 209 mg. methionine per 100-gm. diet did not lead to good growth but was improved equally well by supplements of cystine and methionine (equal sulfur content) or by mixtures of these amino acids in varying proportions (1:2, 1:1, 2:1). The basal diet contained 52 mg.% of protein sulfur and the supplemented diets, 185 mg.%. Greater amounts of cystine and methionine added to the low casein diet to give organic sulfur contents of 229 and 259 mg.% respectively did not increase the protein efficiency of the diets. It is concluded that in the presence of choline, the amounts of the sulfur-containing amino acids and particularly of methionine effective in the promotion of growth may be much less than have been estimated under different experimental conditions by others.

Effect of aureomycin on growth response of rats to various forms of vitamin B_6. HELLEN M. LINKSWILER,* C. A. BAUMANN AND E. E. SNELL. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

In agreement with earlier results, rats fed pyridoxal or pyridoxamine incorporated into an assay diet for vitamin B_6 grew less than rats fed pyridoxine. The addition of 20γ of vitamin B_{12}/kg. of the basal ration did not alter the relative activities of the three forms of vitamin B_6. Aureomycin (50 mg/kg.) increased the growth of rats fed .25 to .75γ of pyridoxine per gram of diet. This increase was less marked at higher levels of the vitamin, but was sufficient to cause over-all errors of 10 to 100% in a bioassay for vitamin B_6. Aureomycin caused a marked increase in the growth of rats fed pyridoxal or pyridoxamine. In the presence of the antibiotic growth due to the three forms of vitamin B_6 was approximately equivalent. Rats fed an assay diet containing 18% of casein and 0.4% of methionine showed linear growth responses to graded doses of vitamin B_6. No preliminary depletion period was necessary. It is postulated that aureomycin may act to prevent utilization or destruction of the growth-limiting nutrient, vitamin B_6, by intestinal microorganisms, thus increasing the amount available to the rat, and that a similar action directed toward other known and possibly unknown food factors may explain in part the growth-promoting effects of antibiotics in crude rations.

Blood dyscrasias in rats resulting from feeding iron salts and their correction by folic acid. ERNEST G. MCDANIEL* AND FLOYD S. DAFT. *Natl. Institute of Arthritis and Metabolic Diseases, National Insts. of Health, Bethesda, Md.*

Weanling rats fed a purified diet containing 8% of casein and approximately 3% of ferric citrate (or an equivalent amount of another iron salt) show growth failure and in 4 to 6 weeks develop marked granulocytopenia with varying degrees of leucopenia. Anemia, also appears but not in all animals. Lower levels of iron salts are effective but the incidence of blood dyscrasias may be reduced. The blood changes are completely prevented or corrected by the administration of folic acid and partially prevented or delayed by feeding higher levels of protein or some combinations of amino acids. Animals receiving ferric citrate excrete more nitrogen in the feces than pair-fed litter-mates receiving the same diet without iron salt.

Evidence for an unidentified growth factor for chicks and turkeys in products of animal origin. JAMES MCGINNIS, L. R. BERG, JOEL R. STERN, J. S. CARVER (introduced by L. C. NORRIS). *Poultry Dept., Washington State College, Pullman.*

Data obtained from feeding trials with chicks and turkey poults indicate that products of animal origin contain an unidentified factor which stimulates early growth. In experiments with Broad Breasted Bronze poults, two basal diets were used, the first containing corn and soybean oil meal, the second containing in addition 10% of fish meal. Both basal diets contained vitamin supplements, including B_{12}. Growth on the second basal, con-

taining fish meal, was better than on the first which had no fish. The addition of the antibiotics, penicillin, terramycin and aureomycin to the first basal diet resulted in a moderate increase in weight gain, whereas addition of these antibiotics to the second basal resulted in a greater increase in weight gain. White Leghorn chicks gained more when penicillin was added to a soy-fish basal than when added to basal without fish. The growth of cross-bred chicks fed a corn-soy diet supplemented with methionine and B vitamins including B_{12} was improved by the addition of fish meal, dried whey, liver meal and especially by fish solubles. Purified casein and additional amino acids were ineffective. These responses were also obtained when sodium proteinate was fed with corn in the basal diet. It is suggested that there exists in products of animal origin, particularly fish solubles, a growth factor which is as yet unidentified.

Effect of vitamin B_{12} and folic acid on glycine toxicity in the chick. L. J. MACHLIN,* C. A. DENTON* AND H. R. BIRD. *Bureau of Animal Industry, U. S. Dept. of Agriculture, Beltsville, Md.*

Day-old chicks from dams fed a vitamin B_{12} low ration were placed on diets consisting primarily of corn and soybean meal for 4 to 6 weeks. Glycine was added at levels of 3, 6, and 9%, with and without the addition of crystalline vitamin B_{12}. The chicks were able to tolerate up to 6% glycine in the presence of B_{12}, whereas 3% was definitely toxic in its absence. In the second experiment, glycine was added at levels of 6 and 9%, and the B_{12} content of the diets was varied. The apparent requirement for B_{12} was increased by the addition of glycine. In the third experiment, a 6% level of glycine was used and B_{12}, folic acid, choline, or a combination of folic acid and choline were added. Folic acid, in the presence of choline, alleviated the glycine toxicity. In the 4th experiment, the diets contained B_{12} and folic acid, alone and in combination, with and without the addition of 6% of glycine. Folic acid again helped to counteract the toxicity, although not as much as did the B_{12}. When both were added, the chicks grew almost as well as those receiving no glycine. The uric acid content of the blood was raised either by glycine or B_{12}. It was raised more by the two in combination than by either alone. Folic acid reversed this effect and decreased blood uric acid.

Absorption of iron from spinach by 6 young women and the effect of beef upon absorption. THELMA J. MCMILLAN* AND FRANCES A. JOHNSTON. *N. Y. State College of Home Economics, Cornell University, Ithaca.*

The objects of the study were to determine the degree to which young women can absorb iron from spinach and whether the presence of beef in the diet mixture might increase the absorption from spinach. The amount of iron absorbed by 6 women was determined for 3 periods of 4 weeks each on *1*) a basal diet containing 6.42 mg. iron/day and including 110 gm. beef for dinner; *2*) the basal diet plus, at dinner with the beef, 120 gm. cooked spinach containing 5.04 mg. iron; *3*) the basal diet plus the serving of spinach for breakfast. No other animal protein was served for breakfast or dinner. The calculations were based on data obtained from 4 subjects. The mean absorption of iron from the spinach for 8 weeks was 13%. The mean daily differences between the total amounts of iron in the diets and that in the feces were for the first period 0.76 ± 0.101, for the second 1.20 ± 0.220, and for the third 1.63 ± 0.147 mg. The difference between the periods when spinach was fed for breakfast and for dinner was not significant; the difference between the basal period and the mean of the periods with spinach was highly significant. The absorption of iron from spinach compared favorably with that from a mixed diet; the presence of beef did not significantly influence the amount absorbed from spinach.

Ultramicromethod for separation and determination of fatty acids on nylon thread. SHU-HUAI MAI (introduced by F. C. BING). *Dept. of Exptl. Medicine, Northwestern Univ. Med. School, Chicago, Ill.*

Nylon thread has been found very suitable for the separation of fatty acids. The following scheme has been worked out for the separation of mixtures containing the straight chained, even numbered, saturated acids from C_8 to C_{18}: *1*) The mixture is first separated into two groups, i.e. C_8–C_{12} and C_{14}–C_{18}, by developing the original mixture on nylon thread (previously extracted with a series of solvents) with a 1:1:1 ethanol:water:acetone mixture. The thread is cut and the two groups are then fractioned further. *2*) The C_{12} acid is separated from the first group by contact with a 1:1 acetone:ethanol mixture. After cutting, the remaining two acids (C_8 and C_{10}) are separated by contact with isooctane. *3*) The C_{14} acid is separated from the second group by redistribution with the original ethanol-water-acetone mixture. After cutting, the two higher homologues (C_{16} and C_{18}) are finally separated with a 1:1:8 mixture of ethanol, water and acetone. The position of the acids on the thread is determined in parallel experiments using bromcresol green as an indicator. The separated acids are extracted with Skellysolve A and estimated quantitatively by Jones' modified monolayer film method. Owing to the solubility of the C_8 acid in dilute mineral acids, it is more conveniently determined colorimetrically with bromcresol green. The working range of the

method is 2–6γ of each acid in the solution applied to the thread. It is planned to apply this method to the study of fat transport in body fluids.

Sudden death in riboflavin deficient dogs. GEORGE V. MANN AND ANNE MCNALLY (introduced by F. J. STARE). *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Adult dogs were fed a purified diet containing all the known vitamins except riboflavin. Seven of these animals seemed to thrive on this diet and became obese. There were no clinical manifestations of deficiency. On the 90 ± 15 day, 7 of the animals collapsed with weakness and vomiting and within a few hours became comatose and died. Convulsions were frequently seen. Saline and dextrose infusions with parenteral riboflavin would prevent death if given immediately after the initial collapse. In this event, treated animals appeared normal in a few hours but without more riboflavin the collapse recurred in a few weeks. Two animals developed anorexia after 5 to 8 weeks on the deficient diet. These animals did not die suddenly but only after developing marked weakness and cachexia. Electrocardiographic changes were marked in all deficient animals. These consisted of S T segment depression and T wave inversion. Terminally the heart rate became slow and the voltage low. These changes were quickly reversible with riboflavin therapy. Animals dying suddenly of riboflavin deficiency did not have fatty livers. The two animals dying of cachexia did have fatty livers. No gross or microscopic lesions could be found at autopsy in those animals dying suddenly.

Relation of 'steatitis' or 'yellow fat' in mink to dietary fats and inadequacy of vitamin E. KARL E. MASON AND G. R. HARTSOUGH.* *Dept. of Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y., and Associated Fur Farms, New Holstein, Wisc.*

Sporadic seasonal outbreaks of 'yellow fat' or 'steatitis' in mink kits during the first few months after weaning have been attributed to botulism, excess protein and deficiency of certain B vitamins. High intake of storage fish and meat products has recently been proposed, and fresh horse meat or liver advocated, for preventing or limiting the disorder (HARTSOUGH AND GORHAM. *Vet. Med.* August, 1949). The symptomatology and pathology closely resemble conditions observed in rats reared from weaning on diets low in vitamin E and high in cod liver oil. Histologic study of tissues from mink dying of steatitis, secured from 3 different ranches, and from mink at pelting season several months after an epidemic, suggest a common etiology for the two conditions. Degeneration of skeletal muscles was prevalent in the acute phase. With acid-fast staining, the altered morphology and pigment (ceroid) deposition in adipose tissue and in macrophages of connective tissue and lymph nodes, especially in the tissues of many mink at pelting time, were usually indistinguishable from those encountered in the rat (MASON, DAM AND GRANADOS. *Anat. Rec.* 94: 265, 1946) under the conditions mentioned. The disorder in mink is attributed to high dietary intake of unsaturated fats in storage fish and meats combined with loss, and general inadequacy, of vitamin E. Dietary control should prevent serious economic losses related to mortality and pelt quality. (Supported by a grant from the Nutrition Foundation, Inc.)

Mechanism of regulation of food intake. J. MAYER AND M. W. BATES (introduced by F. J. STARE). *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

A series of experiments correlating variations of the blood level of various metabolites with food intake has been conducted. Large numbers of white rats (12 to 20) were used for each group so as to be able to detect significant effects while using moderate physiological doses of the substances assayed. Dogs and mice were also used. Solutions of various sugars, amino acids and fat emulsions were administered subcutaneously or intravenously with saline solutions used as controls. Insulin and epinephrine were used to modify glucose levels. It was found that variations of food intake were strikingly correlated to variations of blood glucose and were influenced by other metabolites only insofar as their administration caused direct or indirect variations of blood glucose, with the possible exception of sucrose. This in turn suggests that phosphorylation in the 6-hexose position in the hypothalamus, causing a decrease in ATP and a consequently altered electric state in the neurons, may be the key to the regulation. An additional part of the mechanism, correcting the regulation by successive compensation, is provided by the liver glycogen. While the normal release of glucose from liver glycogen is too slow to follow the diurnal variations of the latter, a lowered liver glycogen may cause a prolonged constant decrease of blood glucose, sufficient to be self-correcting over a period of a few days.

Role of tocopherols in utilization of carotene. AGNES FAY MORGAN AND JOAN W. HEBERT*. *Dept. of Home Economics, Univ. of California, Berkeley.*

Rats given 0.5 mg. alpha tocopherol daily had liver vitamin A increased over the controls when the daily carotene dosage was between 45 and 140 mcg. or total dosage 700 to 2000 mcg. At lower and higher levels of carotene intake tocopherol depressed storage. Vitamin A was found in the intestines of rats only between 30 and 150 minutes

and in the liver only after 90 minutes following carotene dosing. Depression of growth accompanied the increase in liver vitamin A due to tocopherol feeding with either carotene or vitamin A. This is taken to indicate tocopherol-induced storage of vitamin A ordinarily utilized in growth. Adrenalectomized animals receiving one moderate dose, 314 mcg., of carotene in water increased their liver vitamin A above the normal and exhibited vitamin A in the intestine only when tocopherol was given. When 1275 mcg. carotene was given adrenalectomized rats had liver and intestinal vitamin A depressed below that of the normal rats both with and without tocopherol but their intestinal vitamin A content was significently improved by the tocopherol. The absorption and conversion of the larger dose of carotene apparently exceeded the ability of the adrenalectomized animals. In the absence of the adrenal glands carotene transformation occurs but its absorption is impaired and this condition is alleviated by alpha tocopherol.

Nitrogen balance index in the adult rat as affected by diets low in L- and DL-methionine. E. S. NASSET AND JOSEPH T. ANDERSON.* *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

The two isomers of methionine are generally considered metabolically equivalent. The results to be presented show that this may not be true in certain instances. Mature rats were fed amino acid mixture low in methionine by stomach tube. Nitrogen intake was sufficient nearly to maintain nitrogen balance in one set of experiments and approximately half this amount in another set. With full nitrogen intake, that is, at or near nitrogen balance, L-methionine and DL-methionine were equally effective in maintaining nitrogen balance. With only half the nitrogen intake required for nitrogen balance, L-methionine was significantly superior to DL-methionine. At this low level of nitrogen intake it appears that the rat is unable to utilize D-methionine for maintaining nitrogen equilibrium. From the experimental results it is computed that the average requirement of the adult rat for nitrogen balance under these conditions is 3.76 mg. methionine nitrogen, or 40.0 mg. of the amino acid, per day/$kg^{3/4}$.

Dietary protein as a lipotropic agent for cholesterol. RUTH OKEY AND ELIZABETH TURNER.* *Dept. of Home Economics, Univ. of California, Berkeley.*

That methionine, presumably acting through its methyl group, can prevent formation of glyceride fatty livers in rats fed low protein–high fat diets is generally known. The potential lipotropic effect toward fat and cholesterol of variations in protein intake within 'normal' range is less well appreciated. Weanling rats were given adequate synthetic diets containing 1% cholesterol and 13.5% fat but varying amounts of protein (casein and egg albumin). Choline was included at 'low adequate' and 'moderate' levels, 0.5 and 2.5 mg/gm. diet respectively. Average lipid values expressed as milligrams per liver for groups of approximately 12 male rats each follow: low choline with 15% protein, 1876 mg. fatty acid and 493 mg. cholesterol, with 20.2% protein, 1105 mg. fatty acid and 230 mg. cholesterol, with 22.5% protein, 516 mg. fatty acid and 95 mg. cholesterol; moderate choline with 15% protein, 740 mg. fatty acid and 261 mg. cholesterol, with 22.5% protein, 655 mg. fatty acid and 118 mg. cholesterol. Liver lipid data from rats fed similar diets of higher choline content showed little or no further lipotropic effect from the choline but indicated that protein intake might be very important in relation to the use of food cholesterol.

Nutrition and alcoholism. E. O'MALLEY,* V. HEGGIE,* M. TRULSON,* R. FLEMING* AND F. J. STARE. *Dept. of Nutrition, Harvard School of Public Health, and Alcoholic Clinic, Peter Bent Brigham Hosp., Boston, Mass.*

Experiments with rats have shown that voluntary intake of alcohol is greater on marginal diets. Supplementation with massive doses of vitamins caused a decrease in this consumption. We have been interested in extending these studies to humans. To date some 50 chronic alcoholic patients have been studied. Approximately half were given daily massive doses of most of the known vitamins. An equal number were given Placebos. After several weeks some were changed from Placebos to vitamins and vice versa. Depending on the drinking pattern and on other factors, some alterations have been observed. A diminished craving for alcohol has been apparent in many patients when receiving the vitamin supplementation. Most of the patients receiving the vitamin supplementation reported an improved sense of well-being.

Comparative absorption of amino acids from the intestine. ALINE UNDERHILL ORTEN, K. KOIZUMI,* C. J. FRANCE* AND CHARLES G. JOHNSTON. *Dept. of Surgery, Wayne Univ. College of Medicine, Detroit, Mich.*

It has previously been shown (ORTEN, FRANCE AND KOIZUMI. *Federation Proc.* 9: 211, 1950) that the starch column chromatographic technic of Moore and Stein (*J. Biol. Chem.* 176: 337, 367, 1948) can be adapted for use in the separation and determination of phenylalanine, leucine and isoleucine in the intestinal loop contents of the dog. The method has been further developed for preparing intestinal loop contents so that 9 more

amino acids contained therein can be separated and quantitatively determined in 0.1 mg. quantities. These amino acids are tyrosine, glutamic acid, threonine, aspartic acid, serine, glycine, arginine, methionine and lysine. The rates of absorption of certain of the individual amino acids from isolated intestinal loops in the dog have been studied. Distinct differences in the absorption rates have been found. Furthermore there have been observed alterations in the absorption rates of individual amino acids in the presence of other amino acids. (Supported, in part, by a research grant from the National Institutes of Health, Public Health Service.)

Effects of heat on nutritive value of protein hydrolysates with dextrose. LACY R. OVERBY* AND DOUGLAS V. FROST. *Abbott Labs., North Chicago, Ill.*

Amino acid-dextrose solutions were studied with regard to changes in color, fluorescence, and amino nitrogen, disappearance of individual amino acids and loss of nutritive value on autoclaving. Glycine-dextrose model systems followed the pattern described by Friedman and Kline (*J. Biol. Chem.* 184: 599, 1950), of increase in fluorescence before rapid browning occurred. Sulfite increased and extended fluorescence and delayed color formation. Acid hydrolysates of fibrin in dextrose showed initial fluorescence, which decreased on repeated autoclaving. Sulfite delayed both the decrease in fluorescence and extended the color induction period as in the model system. Changes in nutritive value were not correlated with fluorescence, but were clearly related to marked increase in color. Significant decreases in rat-repletion response were noted when *1*) the amino nitrogen showed about a 6–8% decrease, *2*) the fluorescence decreased to a low value following early maximum and *3*) the color increased sharply following induction period. Microbiological assay on heat-darkened hydrolysates with dextrose indicated progressive loss of many essential amino acids. By far the greatest individual loss was in tryptophan. All were regenerated on further acid hydrolysis, except tryptophan. Significantly, the decrease in nutritive value could be largely corrected by tryptophan replacement. This was true up to a point of very marked darkening, at which growth-inhibitory substances appeared.

Weight of adrenal gland as a criterion of ascorbic acid adequacy in the nutrition of the guinea pig. W. H. PFANDER* AND H. H. MITCHELL. *Div. of Animal Nutrition, Univ. of Illinois, Urbana.*

One hundred and twenty guinea pigs ranging in weight from 190–900 gm. were trio-fed on a modified Crampton scorbutogenic diet, supplemented with varying levels of ascorbic acid, for 7 weeks under laboratory conditions. At the end of the experimental period the animals were sacrificed by a blow on the head. Of the criteria of vitamin C adequacy studied (height of odontoblasts, ascorbic acid content of white blood cells, plasma, and adrenals, hemoglobin level, water intake, and weight of the adrenal glands expressed as mg/100-gm. body weight), the weight of the adrenal gland was the most sensitive measure of the ascorbic acid requirement. On adequate levels of ascorbic acid the adrenal glands averaged 60 mg/100-gm. body weight, and as the level of ascorbic acid decreased the adrenal size increased. Histological examination indicates that the increase is largely in the cortex. The enlarged adrenals contained the same percentage of lipid and nitrogen as the normal adrenals. The daily requirement for ascorbic acid, judged by this criterion, is about 0.65 mg/100-gm. body weight for animals weighing less than 400 gm. and 0.55 mg. for animals weighing more than 500 gm. Since the larger animals contain a lower percentage of nitrogen, the requirement of the guinea pig for ascorbic acid seems to be related to the amount of metabolic tissue.

Folic acid, crystalline vitamin B_{12} and penicillin in reproduction in rats. L. R. RICHARDSON AND E. L. RUSSELL.* *Texas Agricultural Experiment Station, College Station, Texas.*

The effect of folid acid, crystalline vitamin B_{12}, and combinations of folic acid and vitamin B_{12}, folic acid, vitamin B_{12} and penicillin, and folic acid and penicillin on reproduction in rats were investigated. The synthetic basal diet contained 30% Labco Vitamin Free casein and was supplemented with adequate amounts of the vitamins required by the rat except those above. Each group contained 15 females and received the experimental diet from weaning. The amounts of vitamins supplied per kg. of diet were: folic acid 1.0 mg., crystalline vitamin B_{12} 25 mcg. and penicillin 10 mg. The number of young observed and percentage weaned respectively in each group were as follows: basal, 146 and 32.2%; folic acid alone, 75 and 66.7%; vitamin B_{12} alone, 107 and 51.4%; folic acid plus vitamin B_{12}, 210 and 70.5%; folic acid, vitamin B_{12} and penicillin, 265 and 70.6%; folic acid and penicillin, 107 and 62.6%. The results obtained with the basal diet and with a combination of folic acid and crystalline vitamin B_{12} were essentially the same as those obtained in an earlier experiment with a similar basal diet and a combination of folic acid and an APF concentrate. When the diet in the earlier experiment was supplemented with folic acid alone only 43.7% of 1028 young were weaned. When it was supplemented with 2.0 gms. of APF concentrate no. 3/kg. alone 43.5% of 783 young were weaned.

Influence of diet on incidence of alloxan diabetes. RICARDO R. RODRIGUEZ* AND WILLARD A.

KREHL. *Yale Nutrition Lab., Dept. of Physiological Chemistry, Yale Univ., New Haven, Conn.*

Although both Houssay and P. Gyorgy have investigated the effect of dietary factors on the early mortality following alloxan injection in rats, certain points of disagreement in their results suggest further work on this problem. Male rats of the Wistar strain, weighing between 100 and 150 gm. were divided into groups and each group fed for one month a particular diet; the diets varied in their content of carbohydrate, protein and fat. They were then injected intraperitoneally with 160 mg. of alloxan/kg. The following results were obtained: *1*) the toxic action of alloxan, evidenced by mortality in the first week following injection, increased in animals fed a high lard or a high coconut oil diet, compared with the group given a high carbohydrate diet. *2*) Ten days following the injection of alloxan the incidence of diabetes in the rats on the high carbohydrate diet was about 50%. *3*) On the other hand, the high lard diet increased the diabetogenic action of alloxan while the incidence of diabetes was diminished on the high coconut oil diet. *4*) No gross evidence of hemoglobinuria was noted in the several groups of rats. *5*) The influence of the isocaloric composition of the diet and the action of tocopherol in alloxan diabetes were studied.

Some chemical and physical properties of guinea pig antistiffness factor. HARRIS ROSENKRANTZ,* ADE T. MILHORAT, ANNE E. MILMAN* AND MILTON FARBER.* *Depts. of Psychiatry and Medicine, Cornell Univ. Med. College, Russell Sage Inst. of Pathology, New York Hosp., New York.*

The application of counter-current distribution and ultra-violet and infrared absorption analyses have permitted fractionation of the antistiffness factor isolated from sugar cane wax. The material (kindly supplied by Eli Lilly & Co.) was separated into steroid and non-steroid fractions by distribution in a methanol-isooctane two-phase system in a Craig apparatus. Characteristic ultra-violet absorption and dry weights were utilized in determining migration while infrared absorption analyses ascertained the relative purity of the components, and were employed in characterizing the molecules. Three fractions were obtained. Fraction A, appeared at the more polar end, and gave an ultra-violet spectrum identical to that published by Wagtendonk. The infrared spectrum resembled stigmasterol more clearly than cholesterol or spinasterol compounds. The ultra-violet spectrum indicated the presence of a diene impurity probably of the ergosterol type. The spectra showed the material not to be identical with ergostanol. Precipitation by digitonin, in addition to infrared analyses, indicated the presence of a beta hydroxyl group. Strong Liebermann-Burchard and mild Rosenheim color reactions were observed. Fraction B appeared to be a mixture of Fractions A and C. Fraction C gave negative color tests and was transparent in the ultra-violet; its infrared spectrum showed unusual absorption bands near 14μ. Fraction C appeared to be free of steroid materials and was toxic in doses of 5γ daily in young guinea pigs.

Effects of feeding unsaturated fatty acids to immature rats with and without tocopherols. RUTH E. RUMERY (introduced by KARL E. MASON). *Dept. of Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

During recent years we have been unable to produce late-lactation paralysis previously observed in weanling rats from stock mothers transferred at parturition to vitamin E-deficient diets containing 18% lard and 2% cod liver oil. Following the same experimental procedure, lard stripped of tocopherols by high-vacuum distillation, comprising 20% of low-E diets, produced no paralysis. Partial hydrogenation of lard during recent years and consequent reduction of unsaturation, rather than increased tocopherol content, may account for the above-mentioned failure. Cod liver oil used as dietary fat in E-deficient diets was well tolerated if fed at 6% but when raised to 10% it produced acid-fast pigment deposition in adipose cells, widespread dystrophy and mortality by 55 days, in 90% of the rats. A 20% level of cod liver oil caused severe paralysis followed by death of all rats by 30 days, and produced anemia, low serum proteins, fatty infiltration and focal necrosis of the liver, and marked pigmentation of fat depots. There was considerable mortality by 60 days of life in rats placed on this diet at weaning but not in those transferred at 30 days of age. Supplements of mixed natural tocopherols (0.85 mgs. daily) prevent all of the changes mentioned. Increased tolerance of young rats for dietary unsaturated fats as maturity progresses, and the ability of tocopherols in enabling the organism to overcome the deleterious effects of such fats, is emphasized. (Supported by a grant from the Nutrition Foundation, Inc.)

Retention of essential amino acids by growing chicken. WALTER C. RUSSELL, M. WIGHT TAYLOR AND WILLIAM A. PRICE, JR.* *Dept. of Agricultural Biochemistry, New Jersey Agricultural Experiment Station, Rutgers Univ., State Univ. of New Jersey, New Brunswick.*

The essential amino acid content of the whole body of the growing chick shows that the following percentages of ingested amino acids were retained: glycine, 45; lysine, 43; threonine, 39; arginine, 38; valine, 38; isoleucine, 37; histidine, 35; methionine, 34; phenylalanine, 30; leucine, 29; and tryptophane, 27. The average retention, 35%,

agreed closely with the value of 33% for total nitrogen retention. Of the total nitrogen in the body of the chick, 54% was accounted for by essential amino acids. In the droppings the following percentages of amino acids, in terms of the quantities ingested, were found: glycine, 14; lysine, 21; threonine, 20; arginine, 11; valine, 19; isoleucine, 18; histidine, 15; methionine, 12; phenylalanine, 14; leucine, 16; and tryptophane, 15. The quantities unaccounted for, and presumably metabolized, averaged 48% and ranged from 36% for lysine to 58% for tryptophane. The pattern of essential amino acids in the body protein of the chick showed a close relationship to that of the food protein.

Metabolic effects of specific amino acid deficiencies. L. T. SAMUELS, H. C. GOLDTHORPE* AND T. F. DOUGHERTY.* *Depts. of Biochemistry and Anatomy, Univ. of Utah College of Medicine, Salt Lake City.*

Young adult male rats were fed diets by stomach tube in which crystalline amino acids furnished the sole source of protein nitrogen. The effect of the absence of either tryptophan, isoleucine, or phenylalanine on the structure and composition of various tissues was then studied. In all cases, absorption of food during the experimental period (3 weeks) was good. The animals on each of the deficient diets went into negative nitrogen balance on an intake of food which maintained a positive balance when all amino acids were present. Yet the excretion of amino acid nitrogen in the urine on the deficient diets was slightly lower than in the controls, rather than higher. Apparently the amino acids were deaminated and converted to fat. This was confirmed by the increased fat in the livers and carcasses of the deficient animals. The serum albumin decreased in isoleucine and tryptophan deficiency but the other serum proteins remained normal. At the same time, liver nitrogen showed only a small decrease. The atrophy of the pancreas and thymus as well as the fatty liver resembled the conditions seen in the tropical nutritional disease, Kwashiorkor.

Influence of vitamin B_{12} and folacin on synthesis of choline and methionine by the rat in vivo and in vitro. A. E. SCHAEFER, J. L. KNOWLES* AND W. D. SALMON. *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn, Ala.*

Weanling rats fed a choline-low basal diet containing thoroughly methanol-extracted peanut meal and oxidized casein failed to gain in body weight unless the diet was supplemented with DL-methionine. The effectiveness of various precursors for synthesis of choline and methionine *in vivo* were measured by gain in body weight, protection against renal damage and total carcass choline. Replacing the DL-methionine and choline supplements with DL-homocystine and various choline precursors, respectively, with and without vitamin B_{12} and/or folacin indicated that vitamin B_{12} and folacin were involved in the synthesis of choline and methionine. When the basal diet was supplemented with homocystine and ethanolamine, methylation of these compounds by methyl from betaine occurred only when the diet was supplemented with vitamin B_{12} and folacin. Glycine and serine or methanol and aminoethanol did not prevent the synthesis of sufficient choline for the support of growth and the prevention of fatal renal hemorrhage in weanling rats. *In vitro* studies were made with liver slices from rats receiving the various deficient diets and from chicks receiving similar diets. The addition of vitamin B_{12} and folacin to substrates containing dimethylaminoethanol resulted in a 2- to 4-fold increase in synthesis of free choline.

Heat processing and nutritive value of milk and milk products. LAWRENCE J. SCHROEDER,* MICHAEL IACOBELLIS* AND ARTHUR H. SMITH. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

The deleterious effect of heat processing on the nutritive value of proteins in foods has provoked considerable comment. This investigation was undertaken to re-evaluate possible nutritive damage to milk and milk products incurred during commercial processing. Nitrogen balances in 4 adult female dogs were the criteria of possible deleterious change. Samples of commercially pooled raw whole milk were frozen, while other samples from the same source were converted into evaporated milk and whole milk powder. These samples were used as the sole protein sources in an otherwise purified diet. After a preliminary adjustment period, isocaloric diets were fed and daily nitrogen intakes kept constant for each dog. The milks and milk products (reconstituted with water) were added to a non-protein basal diet. Raw milk from the original pool was first compared to raw milk from local sources. No differences in nutritive value in these milks were observed. Local raw milk, serving as 'control' milk, was compared with heat-processed milks. No decrease in either biological value or digestibility was seen. Simulation of processing conditions by autoclaving local raw milk produced no observable nutritive damage. The original milk kept in frozen storage likewise showed no decrease in nutritive value of the contained protein. While the interaction of proteins and carbohydrates is known to decrease the nutritive value of proteins, heat processing as used commercially in the preparation of evaporated milk and whole milk powder does not decrease the nutritive value

of the protein constituents under the experimental conditions employed.

Effect of aureomycin on folic acid-citrovorum factor relation in the rat. KLAUS SCHWARZ (introduced by W. H. SEBRELL, JR.). *Natl. Inst. of Arthritis and Metabolic Diseases, National Insts. of Health, Bethesda, Md.*

Twenty milligram % aureomycin HCl in the diet induced growth increases of 25.3 gm. (purified diet) and 15 gm. (stock diet) after 5 weeks. This may be mediated by bacterial synthesis of an essential substance in the intestine. Since gastric juice inactivates citrovorum factor readily at body temperature the rat might depend upon the formation of this principle in the intestine and in tissues. Livers and cecal contents from 2 groups of rats on stock diet (*I*) and on stock diet and aureomycin (*II*) were analyzed for citr. factor (*Leuconostoc citrovorum*) and for total 'folic group' activity (*Streptococcus faecalis*). The cecal contents of *group I* averaged .09 μg. citr. factor (expressed as crystalline citr. factor, Lederle) plus .106 μg. citrovorum inactive PGA derivatives (expressed as crystalline folic acid) per gm. wet weight. In *group II* .22 μg. citr. factor and no citrovorum inactive PGA were found. In addition to this shift from PGA to increased citr. factor animals receiving aureomycin had greatly enlarged ceca. Citr. factor in the cecum was not bound. The homogenized, autolyzed livers yielded 1.27 μg. citr. factor/gm. wet weight in *group I*, and 2.24 μg. in *group II* (77% increase). More than 95% of the total 'strep. faecalis' activity in liver was found as citr. factor; citrovorum inactive PGA and derivatives, if present, were less than 5%. These findings may help to explain the action of aureomycin on hematopoietic disturbances and on growth.

Determination of vitamin B_{12} in animal tissues. B. S. SCHWEIGERT, HAROLD E. SCHEID* AND MONA M. MARQUETTE.* *Div. of Biochemistry and Nutrition, American Meat Inst. Fndn., and Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Experiments on the effect of adding reducing agents and the method of sterilization of the samples and medium were conducted to improve the method of determining vitamin B_{12} with *L. leichmannii* as the test organism. The results obtained when the samples and medium were autoclaved for 10 minutes in the presence of 2 mg. of thioglycollic acid per tube (10 ml. total volume) were comparable to those obtained after Seitz filtration. The activity of several enzyme preparations (trypsin, pancreatin, takadiastase, chick pancreas and bacterial preparations) was investigated for the release of microbiologically unavailable forms of vitamin B_{12} in animal tissues. The values obtained for muscle and organ meats were approximately doubled after these treatments as compared to the values obtained for cold water extracts. The vitamin B_{12} potency of some of these samples was also determined by the rat and chick assay. The range in vitamin B_{12} potency, (expressed as μg/100-gm. fresh weight) determined by the microbiological and animal assays was as follows: beef round, 1.5–2.5; pork loin and ham, 1.0–2.0; lamb leg, 2.0–4.0; beef kidney, 15–30; and beef liver, 30–100. The vitamin B_{12} content of the rat livers determined microbiologically was increased as the amount of vitamin B_{12} fed was increased. The vitamin B_{12} potency of the meat samples calculated from the data on the vitamin B_{12} content of the rat livers was in accord with the potency calculated from the growth data.

Magnesium-potassium antagonism as it affects respiration and paralysis. SUSAN GOWER SMITH. *Duke Univ. School of Medicine, Durham, N. C.*

It has been observed that dogs depleted of potassium by a deficient diet eventually show lowered serum potassium, develop a flaccid paralysis, marked respiratory distress and die of respiratory failure. Likewise, dogs given parenterally excessive amounts of magnesium salts show a lowered serum potassium, develop a flaccid paralysis, marked respiratory distress and die of respiratory failure. Can these two apparently identical results be correlated in any way and if so on what basis? Twenty-five experiments have been performed on 17 dogs in which paralysis was produced by the administration of magnesium sulfate, the dose ranging from 0.15 to 0.38 gm/kg. with an average of 0.27 gm/kg. The magnesium effects wear off very rapidly as soon as its administration is discontinued. Consequently, if potassium salts are given as soon as the magnesium injection ceases the effect if positive would be indistinguishable from the mere withdrawal of the magnesium. Therefore, potassium chloride was injected along with the $MgSO_4$ (10% aqueous solution) at levels of 0%, 1, 3, 5, 7, and 9%. At less than 5% there was little effect, at the higher potassium levels the undesirable respiratory effects are almost completely antagonized and while the paralysis persists it is distinctly less severe at each dose level of magnesium.

Leucine and histidine tolerance in the human. BETTY F. STEELE AND CORINNE B. LE BOVIT (introduced by L. A. MAYNARD). *School of Nutrition and New York State College of Home Economics, Cornell Univ., Ithaca, N. Y.*

Human subjects were fed aqueous solutions of leucine or histidine. The amino acid administered was determined in the urine, plasma, and formed elements of the blood at intervals up to 4 hours after administration of the test dose. Microbiologi-

cal methods were used throughout. Renal clearance values for leucine were 1 ml. or less even when the test dose raised the level of leucine almost 9-fold over the fasting plasma level. The peak value was reached half an hour after administration of up to 7.5 gm. of L-leucine, then dropped sharply and approached the fasting level at 4 hours. When the subjects were fed 10 gm. of leucine the plasma values remained elevated for a longer period, but approached fasting levels at the end of 4 hours. The leucine content of the formed elements paralleled that of the plasma. Individual subjects varied in their responses to the test doses of L-histidine. Clearance values were 3 ml. or more. Some subjects exhibited the sharp rise and fall of plasma histidine found with leucine, others did not. The histidine content of the formed elements tended to remain elevated for the entire 4-hour period. It is concluded that both leucine and histidine are efficiently reabsorbed by the kidney, although the actual excretion of histidine is much higher than the excretion of leucine. No sharp renal threshold was reached for either amino acid.

Vitamin B_{12} in growth, reproduction and lactation. BARNETT SURE. *Dept. of Agricultural Chemistry, Univ. of Arkansas, Fayetteville.*

Results on the influence of the addition of 0.1 μg. daily of crystalline vitamin B_{12} on growth of the albino rat, during 10 weeks of the post-lactation period, are as follows: As a supplement to a ration containing 25% proteins furnished as low fat soybean flour: 15 males, 20% increased growth; 15 females, 7.2% increased growth. As a supplement to a ration containing 28.2% alpha-soybean protein: 18 animals, 38.5% gain in body weight. As a supplement to 25% proteins in ration furnished as low fat cottonseed flour: 18 animals, 38% increase in body weight. As a supplement to a ration containing 94% as the breakfast food, Cerevim, the addition of 0.1 μg. daily of vitamin B_{12} for growth, 0.2 μg. for reproduction, and 0.5 μg. for lactation, resulted in marked improvement in reproduction, and lactation efficiency was increased from 37 to 73%. Three generations of animals with excellent growth records were obtained when such vitamin B_{12}-supplemented ration furnished all the B vitamins, proteins and minerals. The addition of 1% APF from a fermentation product of aureomycin to the Cerevim ration also produced very favorable effects on reproduction and lactation, and 3 generations of healthy growing animals have been obtained on the latter ration.

Serum flavin levels in riboflavin deficiencies. KAMDHORN SUVARNAKICH AND PATRICIA STEVENS (introduced by F. J. STARE). *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Adult dogs and juvenile monkeys were fed purified diets deficient in riboflavin and their serum riboflavin levels measured bi-weekly. There was an immediate diminution of free riboflavin plus flavin-mononucleotide to zero or almost zero levels in 7 to 14 days. This was followed in about 2 to 3 weeks' time by a decrease in flavin-adenine-dinucleotide to about half the normal level. Thereafter, the serum flavin levels remained nearly stationary. The weights of 7 of 9 dogs increased steadily up to the time of death, which took place suddenly and between 10 to 15 weeks after the beginning of the deficient diet. These animals, in most cases, appeared clinically normal until a few hours before death. In 2 dogs anorexia and weight loss began 5 to 7 weeks after beginning the deficient diet. Continuation of the deficient diet did not further lower the serum riboflavin levels. Supplementation with riboflavin produced marked improvement in physical condition and appetite but rise in serum levels of riboflavin was delayed several weeks. Young Cebus monkeys on the riboflavin deficient diet uniformly showed a weight loss with cachexia. Serum levels of flavins reached a minimum after 3 to 4 weeks and remained there until death. Supplementation with 30–60 μg. of riboflavin per day produced a spectacular clinical recovery, but the serum flavin levels remained at the minimal levels for 10 weeks after supplementation was begun. The experiments indicate a limitation in the diagnostic value of serum riboflavin values if the degree of riboflavin deficiency is severe.

Contrasting effects of antibiotics in diets low in vitamin A or in members of the vitamin B complex. R. W. SWICK,* HWA LIH* AND C. A. BAUMANN. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Penicillin, aureomycin and streptomycin stimulated the growth of rats receiving limiting amounts of thiamine, riboflavin or pantothenic acid as supplied in bioassays for these vitamins. The increases in growth due to the antibiotics equalled those observed when the vitamin content in the absence of antibiotic was doubled or trebled. The effectiveness of the antibiotic depended on the vitamin deficiency: penicillin was best in thiamin deficiency; in pantothenic acid deficiency aureomycin and streptomycin were superior to penicillin. In the vitamin A assay, on the other hand, there was no general increase in growth due to antibiotics, although penicillin appeared to decrease the number of failures due to respiratory trouble when borderline levels of the vitamin were fed. The results suggest that antibiotics may improve growth by 2 different mechanisms: they

may prevent microorganisms in the digestive tract from absorbing a limiting vitamin and thus withholding it from the host; or they may diminish the activity of organisms that cause disease.

Nutritional status of selected population groups in Oregon as determined by food habits studies and biochemical tests on the blood. CLARA A. STORVICK, MILICENT L. HATHAWAY, RUTH M. NITCHALS,* BERNICE SCHAAD,* RUTH E. COFFEY* AND MARY B. DEARDORFF.* *Nutrition Research Lab., Home Economics Experiment Station, Oregon State College, Corvallis.*

Native born and reared children in two geographic regions were included in a study on food habits and biochemical tests on blood. The records of food intake were evaluated for each of 8 nutrients: calcium, iron, protein, vitamin A value, ascorbic acid, thiamine, riboflavin and niacin. Levels of intake were classified as adequate, borderline or inadequate when compared to the National Research Councils recommended allowances. Over 60% of the children had dietaries adequate in all of the nutrients except iron and ascorbic acid. Less than 10% of the children had diets classified as inadequate in protein, vitamin A value, thiamine, riboflavin and niacin. No regional, county or age differences in the nutritive values of the dietaries were observed. Blood samples obtained by finger puncture were analyzed for serum vitamin A, serum carotene, serum ascorbic acid, hemoglobin, hematocrit value and plasma protein using the micromethods of Bessey and Lowry. By means of analysis of variance, regional differences were found in values for serum vitamin A, hemoglobin and hematocrit value; values for those constituents for the Central Oregon children were higher than for children living in the Coast Region. Regional differences were not found in values for serum carotene, serum ascorbic acid and plasma protein.

Effect of dietary carbohydrate on amebic infections in the guinea pig. D. JANE TAYLOR AND JOSEPH GREENBERG (introduced by JAMES M. HUNDLEY). *Natl. Insts. of Health, Natl. Microbiological Inst., Bethesda, Md.*

It was reported previously (Taylor *et al. Am. J. Trop. Med.* 30: 817, 1950) that the percentage of induced infections of *Endamoeba histolytica* in the guinea pig was 75% when the animals were fed a stock ration versus 41% with stock guinea pig pellets. A modified dry diet (50% dry skim milk, 25.7% sucrose, 17.8% alfalfa leaf meal, 4% salts, 2.5% fat and essential vitamins) increased the infectivity rate to 96%. The effects of the modified diet and a synthetic diet (ROINE AND ELVEHJEM *Proc. Soc. Exper. Biol. & Med.* 73: 308, 1950) on the amebic infection in the guinea pig have been compared. When the carbohydrate of the synthetic diet was sucrose or lactose the intensity of the infection and the death rate were comparable with those of the modified diet. When the carbohydrate was starch the intensity of the infection was significantly lower without changing the death rate. This indicates that starch had an inhibitory effect on the amebic infection. Additional data on other carbohydrates are presented.

Tolerance in rabbits to bacterial pyrogens. DAVID M. TENNENT* AND WALTHER H. OTT. *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Studies have been made of factors influencing the tolerance produced in rabbits by injection of bacterial pyrogens, with special emphasis on the size of the dose and the time required for recovery of normal febrile response. The tolerance developed and the recovery time were found to be functions of the amount of pyrogen given. The effect was produced by a single dose. With a dose sufficient to cause a 0.6°C. temperature rise, a rest period of one week was not always long enough for recovery of normal response.

Nitrogen balance studies in rats fed diets with and without added glycine. W. R. TODD AND ELLEN TALMAN.* *Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.*

The experiments herein reported were designed to test further our earlier contention that feeding rats diets containing added glycine stimulates adrenal cortical activity causing enhanced glyconeogenesis during an ensuing fast (W. R. TODD AND ELLEN TALMAN, *Arch. Biochem.* 22: 386, 1949). Urinary nitrogen excretion should be increased if body protein is undergoing conversion to carbohydrate at an accelerated rate. In balance studies with rats it has been demonstrated that animals fed a synthetic diet containing 10% glycine for 2 days have about a 40% greater negative nitrogen balance during an ensuing 24-hour fast than animals fed the diet without added glycine. This amounts to around 80 mg. of extra nitrogen for a 250-gm. animal during the 24-hour fast. About 80% of the extra urinary nitrogen occurs as urea. The creatinine excretion is depressed slightly and uric acid excretion increased. These are findings reported by others to be associated with increased cortical activity. The creatine excretion is increased 5- to 10-fold which fits in with the assumption of increased tissue catabolism and the well-known fact that the conversion of creatine to creatinine is a slow process. The data uphold the contention that feeding rats diets containing 10% glycine does bring about increased tissue catabolism which would be an essential part of our original hypothesis of enhanced glyconeogenesis during a fasting period following such diets.

Interrelations between choline and vitamin B_{12} in the mouse. JOHN J. TRAVERS* AND LEOPOLD

R. CERECEDO. *Dept. of Biochemistry, Fordham Univ., New York City.*

The greater resistance of the mouse to choline deficiency and the involvement of vitamin B_{12} in the synthesis of methyl groups and choline led to a study (microbiological) of the vitamin B_{12} content of mouse and rat tissues. Under all conditions mouse liver contained more vitamin B_{12} than rat liver. In each species lowest values were obtained with a sulfasuxidine-iodinated casein diet which, after 5 weeks, produced values for mouse liver comparable with those of stock rats, and a 6-fold increase over those of rats similarly treated. A vegetable protein diet caused a temporary decrease in each species, followed by an increase. Even after 8 months, mouse liver contained as much vitamin B_{12} as those of young stock mice, and more than those of young stock rats. Thus, as with choline, younger animals need an exogenous supply of vitamin B_{12}. Since this vitamin is involved in the synthesis of methyl groups and the transmethylation process, it appears that the resistance of the mouse to choline deficiency can be related, in part, to its vitamin B_{12} content. The data are also in line with our inability to produce in mice, in contrast to what has been observed in rats, tumors by choline deficiency; with the recent report on the procarcinogenic effect of vitamin B_{12} in rats; and with the greater incidence of spontaneous tumors in mice. (Supported by a research grant from the National Institutes of Health, Public Health Service.)

Analysis of diet records. M. F. TRULSON* AND F. J. STARE. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Diet records kept or prepared by the individual have been one of the most widely used methods for collecting nutritional data on population groups and for assessing the intake of individual subjects, particularly of school children. The length of time which these records should be kept to establish a reliable index of intake has never been adequately established. We have observed a gradual daily decrease of mean intake values of most nutrients, the longer a dietary survey is conducted, also a lowering of correlation coefficients the further apart the days are correlated. Four surveys involving 7-day dietary records starting on Monday, Tuesday, Wednesday and Friday have been studied. The mean values of these dietary surveys, according to days on which they were started and the correlation among various days, will be discussed.

Effect of diet composition and activity on food intake and body composition. JOSEPH J. VITALE,* JEAN MAYER* AND D. MARK HEGSTED. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Adult, female albino rats were housed individually in small mouse cages so as to minimize energy expenditure while control animals were housed individually in larger cages. Four groups of 6 animals each were used in the 2 sets of cages and were fed on the following 4 diets: normal diet, high protein, high fat, and high fat-low protein. Food intakes were recorded daily during a 3-month period. The animals were then sacrificed. Body analyses were performed for fat and protein. Regardless of the activity restriction, the animals on the high fat and the high fat-low protein diet had a greater food intake and showed slightly more weight gains. There was no difference between the affects of these 2 diets in the restricted groups; however, the unrestricted animals fed the high fat-low protein diet ate significantly more than all of the groups. The dietary intake and activity restrictions had no influence on percentage of body protein. When the amount of glucogenic material in the diet was high, restrictions of activity greatly influenced the amount of fat deposited. However, fat deposition was not affected when the concentration of glucogenic components was low. The caloric concentration of the high fat diets was not responsible for the results noted, for when such diets were diluted with cellu-flour to bring the caloric concentration down to that of the normal and high-protein diets, the same effects were noted.

Growth effect of various antibiotics on baby pigs fed synthetic rations. R. C. WAHLSTROM* AND B. CONNOR JOHNSON. *Div. of Animal Nutrition, Univ. of Illinois, Urbana.*

A synthetic 30% alpha-protein milk ration was supplemented with all known factors and fed to groups of 2-day-old pigs in 3 experiments. The addition of aureomycin or of chloramphenicol at 100 mg/kg. of dry matter consumed caused a significant increase in average daily gain over the basal group and also an increased caecal size. The addition of aureomycin to the diet of pigs not receiving vitamin B_{12} did not prevent the symptoms of vitamin B_{12} deficiency. Injection of crystalline vitamin B_{12} to these pigs after 49 days on a deficient diet caused a significant reticulocytosis as well as an increase in average daily gain and feed efficiency. Chloramphenicol decreased the number of coliform bacteria present in the feces during the initial stages of the experiment; however, by the end of 3 weeks this effect was no longer apparent. Aureomycin, streptomycin and penicillin appeared to have no effect on coliform, lactobacilli or yeast cell counts. Sulfathalidine fed at a level of 2% of the diet caused a sustained significant reduction in coliform bacteria present in the feces. The results (weight gains and fecal bacteria data) of these experiments will be reported as well as the growth effect of condensed fish solubles, grass juice concentrate and vitamin

B_{12} on baby pigs fed the same 'synthetic milk' diet.

Effects of riboflavin and pantothenic acid on the conjugation and detoxification of 2-aminofluorene in the dog. ARTHUR W. WASE* AND JAMES B. ALLISON. *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.*

Excess riboflavin in the diet protected animals from the damaging effects of the carcinogen, 2-acetylaminofluorene. This excess vitamin resulted in an increased excretion of a conjugated form of 2-aminofluorene and of non-volatile phenols which are believed to be detoxified products of the carcinogen. When the free base (2-aminofluorene) was fed only a small amount was normally conjugated. This conjugation was increased markedly in the presence of 10 and 100 times a normal riboflavin intake. It was demonstrated also that increasing the pantothenic acid content of the diet to 100 times the amount considered adequate would raise the excretion of the conjugated form. The increase occurred in the presence of a low but normally adequate riboflavin intake. If the dog was saturated with riboflavin by feeding an excess of the vitamin the conjugation of the free base was at a maximum and increasing pantothenic acid above normal had little effect. These data suggest that pantothenic acid in the diet could effect the acetylation of other amines. It was demonstrated that panthenol (Roche) fed to people would increase the acetylation of sulphanilamide by 5%. The use of these vitamins for detoxification of various amines in animals is being studied further.

Influence of protein and choline on copper absorption. PHILIP L. WHITE,* D. MARK HEGSTED AND J. MAYER.* *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

White Leghorn cockerels were employed in the study of factors influencing the absorption of copper. The chicks were maintained on purified diets with varying concentrations of copper sulfate, choline and protein. Alterations in the course of growth and liver-copper concentration were used as indices of copper absorption. Choline was found to increase the deposition of copper in chicks fed 10% casein diets, whereas higher proportions of protein decreased the toxicity of extra copper even in the presence of choline. The plumage of those birds receiving additional copper was much superior in appearance to the normal or low copper control chicks. The fact that abundant feather production was maintained in spite of inhibited tissue growth suggests that copper influences preferentially certain aspects of protein utilization. The increased absorption of copper in the presence of choline may be due to the formatioe of complex copper-choline salts. Two such salts of choline and copper chloride have been prepared and characterized chemically.

Microbiological evaluation of protein quality: I. A colorimetric method for determination of growth of *Tetrahymena geleii* W in protein suspensions. HAROLD H. WILLIAMS AND MARY E. ANDERSON.* *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*

A method is described for the estimation of the growth of the protozoan, *Tetrahymena geleii* W, in protein suspensions. The procedure involves a colorimetric determination of the red triphenylformazan formed by the reduction of the colorless 2,3,5-triphenyltetrazolium chloride, a reaction effected by the respiratory enzymes of the organism. The reaction is allowed to proceed for 4 hours at 25°C. and is stopped, after this incubation period, by the addition of mercuric chloride. The insoluble formazan is separated from the culture solution by centrifugation and is then dissolved in acetone. The optical density of this acetone solution, as measured spectrophotometrically, has been shown to be proportional to the number of organisms (volume of culture) treated. The colorimetric procedure has been used in obtaining dose-response curves of the organism to the 10 'essential' amino acids—arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The procedure has also been successful in reflecting the response of *T. geleii* to graded nitrogen levels as supplied by soy bean oil meal, whole egg, casein, or animal liver meal. However, no attempt was made to evaluate these proteins numerically, as the criteria for assigning such values have not been definitely established for this organism. (Supported by a grant from the Herman Frasch Foundation to Cornell University.)

Histidine deficiency and liver enzyme activity. J. N. WILLIAMS, JR. AND JAMES W. BOTHWELL (introduced by C. A. ELVEHJEM). *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

In a previous study in which the effects of a methionine deficiency upon rat liver enzyme activity were studied, it was reported that succinic dehydrogenase activity was decreased somewhat, that xanthine oxidase was completely removed and that endogenous respiration was practically unaffected (*Proc. Soc. Exper. Biol. & Med.* 72: 386, 1949). This investigation has been extended to a study of the effects of a histidine deficiency upon the same liver enzymes with the inclusion of choline oxidase. As in the methionine studies, a force-feeding procedure was undertaken to rule

out effects due to differences in food consumption. One group of rats was force-fed a complete ration in which protein was supplied as an amino acid mixture. Another group was force-fed the same ration except that histidine was omitted. After 2 weeks of the feeding regimen, the animals were sacrificed for enzyme determinations. As contrasted to the effects of a methionine deficiency, the histidine deficiency caused very few changes in liver enzyme activity. Liver xanthine oxidase, which disappeared with methionine deficiency, was not decreased at all with the histidine deficiency. Similar results were obtained with choline oxidase and succinic dehydrogenase. Endogenous respiration, however, appeared to be decreased somewhat with the histidine deficiency. A histidine deficiency was strongly evident among the animals fed the histidine-free ration as shown by over 50% mortality of that group after the first week of feeding.

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

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(An asterisk following an author's name indicates "by invitation")*

Role of L-methionine in virus propagation. W. WILBUR ACKERMANN (introduced by THOMAS FRANCIS, JR.). *Dept. of Epidemiology and Virus Lab., Univ. of Michigan, School of Public Health, Ann Arbor.*

An experimental system is described for the study of the propagation of the PR8 strain of influenza type-A virus in the flasks of a Warburg apparatus. The host-cells are those of the chorioallantoic membrane and are maintained in a modified Simms solution. In this system graded concentrations of DL-methoxinine are found to produce a proportional inhibition of the propagation of the virus. This inhibition can be completely blocked by DL-methionine. As increasing amounts of this amino acid are added in the presence of DL-methoxinine, there is produced a corresponding response in the rate of synthesis of the virus. DL-ethionine and DL-methionine sulfoximine are found to act in much the same manner as DL-methoxinine. DL-methionine sulfoximine is 100 times more effective an inhibitor than DL-ethionine or DL-methoxinine. The action of the inhibitors can be blocked by L-methionine but not by D-methionine. Betaine, choline, cysteine and creatinine are inactive when tested for their ability to nullify inhibition by ethionine. DL-homocysteine does partially block the inhibition by both methoxinine and ethionine. These inhibitors do not act by destroying the virus or the host-cells or by preventing infection of the cells, but rather by interference with the synthesis of the virus.

Growth curve of poliomyelitis virus in mice. JOHN D. AINSLIE (introduced by GORDON C. BROWN). *Dept. of Epidemiology and Virus Lab., School of Public Health, Univ. of Michigan, Ann Arbor.*

There is a need for a quantitative description of the pathogenesis of poliomyelitic infection in mice. This could add definitive information for the elucidation of the host-parasite relationship, and would be of immediate practical value, for example, in evaluating the results of chemotherapy for which the infection in the mouse is frequently used as a preliminary screening procedure. Using the Lansing strain of poliomyelitis virus it was found that maximal titers of virus were attained within 18–24 hours after inoculation, which was 24–30 hours before the onset of the first and maximal rate of paralysis. The virus content of the CNS then remained fairly constant for at least a week. Although the titer of virus was greatest in the spinal cords of paralyzed mice, there was little difference between the titer of their brains and the brains and cords of non-paralyzed mice. Confirmation that a high titer of virus is achieved rapidly was obtained when the brains and cords of 1000 mice were harvested 48 hours after intracerebral inoculation, at which time only 14% were paralyzed, and the resulting pool was found to have a very high titer. This procedure of collecting a pool could eliminate the delays consequent on irregularities in the incubation period and in the morbidity, as well as the loss of mice by death, which are inherent disadvantages of the usual method of collecting CNS only from paralyzed mice.

Response to second Schick test in individuals remaining positive after one Schick test. J. S. ALTMAN* AND GEOFFREY EDSALL. *Epidemic Disease Control Unit no. 4, Great Lakes, Ill. and Dept. of Microbiology, Boston Univ. School of Medicine, Boston, Mass.*

In the course of a study on the immunizing efficacy of small doses of diphtheria toxoid, subjects for the study were selected by the performance of two successive Schick tests, on the assumption that those found positive on both tests would be true immunes. Because of a contrary observation by Dr. Kenneth Goodner, this hypothesis was submitted to a test. Out of approximately 300 students at a Navy electronics school, 82 subjects were found whose antitoxin titer prior to testing was < 0.001 U/ml. and who remained available throughout this phase of the study. Thirty of the 82 showed antitoxin titers >0.003 U three weeks after the first Schick test. Twenty-five of the remainder showed a similar rise after the second Schick test. The significance of these findings will be commented upon.

Tuberculin reaction in relation to local reaction to BCG vaccine in initially vaccinated and in

previously vaccinated persons. JOSEPH D. ARONSON AND M. MCGETTIGAN.* *Univ. of Pennsylvania, Henry Phipps Inst., Philadelphia.*

The local reaction to old tuberculin in a group of 3,510 adults not previously vaccinated with BCG vaccine was correlated to the simultaneous but separate injection of BCG vaccine. The intensity and character of the reactions were studied 48 hours later in order to determine the frequency of exaggerated inflammatory reactions (Koch phenomenon) in relation to the tuberculin reaction. The Koch phenomenon was observed in 7.5% of those who failed to react to 0.1 mg. old tuberculin, in 82.5% who showed a slight positive reaction to tuberculin, and in 42.5% of those who gave a doubtful reaction to tuberculin. The reactions to 0.1 mg. old tuberculin and to BCG vaccine were compared in a group of 99 persons vaccinated one year previously and who gave a slight, doubtful or negative reaction to tuberculin. When revaccinated with BCG vaccine, a Koch phenomenon was observed in 55% of those who were negative to 0.1 mg. old tuberculin, in 82.9% of those who were slightly positive to tuberculin and in 71.4% of those who gave a doubtful reaction to tuberculin. Of 46 persons who failed to react to 0.1 mg. old tuberculin, 21 or 45.7% reacted to 1.0 mg. old tuberculin, while 37 or 80.4% of the same group showed an exaggerated inflammatory reaction to BCG vaccine. Accelerated tubercle formation was found in almost all of those who were vaccinated one year previously when revaccinated with BCG vaccine.

Effect of chemical agents on phage receptors of Phase II *Sh. sonnei*. GUY T. BARRY AND WALTHER F. GOEBEL. *Rockefeller Inst. for Med. Research, New York City.*

Because different preparations of the somatic antigen obtained from Phase II *Sh. sonnei* exhibit variations in their ability to inhibit T_3, T_4 and T_7 bacteriophages, a study has been made of the effect of chemical agents on the phage adsorption properties of intact bacterial cells. A comparison of the rates of virus adsorption on live and chemically killed bacteria served as the criterion for the destruction of phage receptors. If the adsorbtive rates are the same, it can be assumed that no alteration of the receptor has occurred during the chemical manipulation of the microorganisms. Among the various reagents being studied are formalin, phenol, ethylene oxide and certain antibiotics. Thus it has been found that when Phase II *Sh. sonnei* are killed by heat or by formal[1] in the rate of adsorption of T_7 is unaffected, whereas that of T_3 is greatly reduced. The results thus far obtained indicate that the type specific somatic antigen of the Phase II organism, as it occurs on the cell surface, appears to be quite labile and can be altered in so far as its ability to adsorb phage is concerned without detectable alteration in its antigenic or specific serological properties.

Purification and characters of the virus of avian erythro-myeloblastic leucosis. J. W. BEARD, DOROTHY BEARD,* EDWARD A. ECKERT,* D. G. SHARP* AND T. Z. CSÁKY.* *Dept. of Surgery, Duke Univ. School of Medicine, Durham, N. C.*

Avian erythro-myeloblastic leucosis is a virus-induced neoplastic disease transmissible experimentally by transfer of filtered plasma to susceptible chicks. Though the condition is readily passed in this way, a high concentration of virus in the plasma is not indicated by the relatively low incidence of positive inoculations (60% with 10^{-1} ml. plasma, 12% with 10^{-121} ml. plasma). Nevertheless, a particulate component, present in relatively large amounts in the plasma of diseased birds, has been isolated by centrifugal procedures. No evidence of this component has been seen in normal chicks or chicks refractory to the disease. Particles of similar character have been obtained also from thoroughly washed primitive blood cells occurring in the disease. Electron micrographic studies have been made of the morphology of the apparently sperm-shaped particles in purified preparations. Serological studies have failed to reveal relationship with other viruses, especially with the Newcastle disease virus which the particles resemble to some extent. The relationship of the particles to the virus with respect to infectious properties has been determined by recently developed procedures for titrating the virus.

Antigen-antibody reactions in agar II. Diffusion coefficients of antigens. ELMER L. BECKER, J. MUNOZ, CLAUDE LAPRESLE AND LEON LEBEAU (introduced by KENNETH ROBBINS). *Dept. of Bacteriology and Allergy Unit, Univ. of Illinois College of Medicine, Chicago.*

In the Oudin serum technique we have derived an equation relating the distance moved, x of the precipitate in time, t, to the antibody concentration Ab, antigen concentration Ag_o, diffusion coefficient of the antigen, D, and the ratio of antigen to antibody at the equivalence point, R'. The equation holds quantitatively only for the limiting case of low concentration of antibody and high concentration of antigen as expected from the assumptions made in its derivation. Under these conditions and using the R' value determined by the conventional quantitative precipitin technique the following values for $D_{20} \times 10^{-7}$ were obtained of 5.9, 5.8 and 6.8 for crystalline bovine serum albumin (B.S.A.), horse serum albumin (H.S.A.) and egg albumin (Ea), respectively. The necessity of knowing R' limits the use of (1) to immunochemically homogeneous

systems. From the first equation mentioned a relationship can be obtained by means of which D can be calculated without the necessity of knowing R′. By means of this second relationship values for ($D_{20} \times 10^{-7}$) obtained for B.S.A., H.S.A. and Ea were 5.8, 5.6 and 7.2. The possible scope and limitations of the method will also be discussed.

Analysis of specific precipitates for distinctive constituents of the antigen. SAM M. BEISER* AND ELVIN A. KABAT. *Depts. of Neurology and Bacteriology, Columbia Univ., College of Physicians and Surgeons, and Neurological Inst., New York City.*

An estimate of the purity of a given antigen may be obtained by analyzing for one of its characteristic constituents in a specific precipitate formed with excess antibody. Since the Type II pneumococcal capsular polysaccharide (SII) was shown by Record and Stacey to contain rhamnose, the colorimetric methylpentose method of Dische and Shettles was used to analyze dissolved specific precipitates. The ratio of methylpentose color value to nitrogen of the antibody portion of specific precipitates was determined on rabbit SIII anti-SIII and Friedlander B-anti-Friedlander B and on human pneumococcal C-anti-C and SIII-anti-SIII precipitates, since these specific polysaccharides gave only traces of methylpentose color. Ratios of methylpentose color to antibody N were 0.041 and 0.031 for the rabbit and human antibody globulins, respectively. Analyses of SII-rabbit anti-SII and SII-human anti-SII precipitates showed, after correction for methylpentose color due to antibody, that 94 and 97% of the methylpentose of the SII was precipitated. Addition of excess cross-reacting rabbit anti-Friedlander B to SII precipitated 99% of the methylpentose of the SII. Since the Friedlander B polysaccharide contains no methylpentose, the cross reaction with SII must be due to structural similarities of the other constituents of the molecules. The ratios of methylpentose to antibody N and the color values with the Folin-Ciocalteau tyrosine reagent for human antibody globulins are identical with those found for normal human gamma globulin.

Immunologic response to vaccination with inactivated cowpox vaccine. ABRAM S. BENENSON AND C. HENRY KEMPE (introduced by G. EDSALL). *Second Army Area Med. Lab., Ft. George G. Meade, Md. and Dept. of Pathology and Bacteriology, Tufts College Med. School, Boston, Mass.*

Previous studies have shown that inactivated cowpox vaccine may produce any type of morphological reaction in previously vaccinated subjects. In order to determine whether such reactions produce a 'boost' in immunity, blood was drawn before and 3 weeks after vaccination of 48 Tufts College medical students with inactivated vaccine, and of 47 with active vaccine. Sera were examined by complement-fixation and hemagglutination-inhibition tests. With inactivated vaccine, all subjects presented immediate reactions with varying morphology; one very allergic asthmatic subject showed a significant rise only with the complement-fixation test; all other subjects showed no significant change in titer with either serological technique. With live virus; 3 students developed vaccinia, 17 vaccinoid reactions and the remainder immediate reactions; the expected serological response was manifested by both procedures; and rises in titer were present with all forms of immediate reaction. One month after original vaccination, 49 students who had received inactivated vaccine were revaccinated with potent material. Two vaccinia and 21 vaccinoid reactions occurred in a total of 49 subjects, essentially the same distribution as observed in the actually vaccinated group. The lack of significant difference in resistance to infection confirms the serological implication that no immunity resulted from the exposure to and reaction from inactivated material and that this reaction is a manifestation of skin sensitization and does not confer immunity unless live virus is employed. With live virus, the immediate reaction observed may represent a combination of sensitivity reaction and abortive infection.

Prevention of paralytic poliomyelitis in monkeys with human immune globulin. DAVID BODIAN. *Poliomyelitis Lab., Dept. of Epidemiology, Johns Hopkins Univ., Baltimore, Md.*

Gamma globulin, from the American Red Cross plasma pools, neutralizes all three known poliomyelitis virus types (*Proc. Soc. Exper. Biol. & Med.* 72: 259, 1949). In rhesus inoculated in calf muscles with 10,000 intracerebral PD_{50}s of viruses of low infectivity by this route (Frederick, Leon, or Lansing), 2 ml. of gamma globulin per kg. in opposite calf muscles at the same time, reduced paralysis due to intramuscular immunization from 10/35 (control rate) to 0/35. Under the same conditions, but with virus of high intramuscular infectivity (MV virus), the paralytic rate was reduced from 14/24 to 3/24. Paralysis was also prevented when gamma globulin was administered 3 days before or 3 days after intramuscular virus injection. Lansing antibody due to the gamma globulin could be detected in low titer in the serum at least as long as 17 days following administration of 2 ml/kg., and 6 (but not 8) weeks after giving 10 ml/kg. In monkeys immunized with Lansing virus (3 times in 4 weeks), with gamma globulin

given concomitantly each time, as described above, antibody levels one week after the last injection were equal to those of control monkeys receiving active virus alone (1:2000). Both groups of monkeys subsequently resisted intracerebral challenge with 1000 PD_{50} of Lansing virus. In orally inoculated cynomolgus monkeys, receiving concomitant intramuscular gamma globulin (10 ml/kg.) paralysis appeared to be prevented, but control paralytic rates were too low to be conclusive.

Allergenic properties of erythrogenic toxin of *Streptococcus hemolyticus*. DANIEL A. BOROFF. *Pasteur Inst., Paris, France.*

In the course of our studies of the erythrogenic toxin of the hemolytic streptococci it was observed that intracutaneous injections of sub skin testing dose of this toxin induced a heightened sensitivity in the skin of the rabbits to subsequent injections of the same amount of this toxin. This sensitivity manifests itself 24 hours after the initial injection by a large wheal and erythema. Subsequent injections only accelerate the reaction. All parts of the skin of the prepared rabbit appeared to be sensitive. The reaction seemed to be specific as the rabbits failed to react to substances other than streptococcus toxin. Streptococcus toxin prepared by us as well as the toxin obtained from other sources possessed this allergenic property. The rabbits remained sensitive for at least 3 months after the initial injection. The rapidity of the allergic response rules out the possibility of its being the Arthus phenomenon. That it is not the Shwartzman phenomenon is evidenced by the failure of the rabbits to react to bacterial substances injected intravenously. All rabbits showing skin sensitivity also showed marked kidney damage not observed in normal controls.

The effect of DL-ethionine on poliomyelitis virus growth in tissue culture. GORDON C. BROWN AND W. W. ACKERMANN. *Dept. of Epidemiology and Virus Lab., School of Public Health, Univ. of Michigan, Ann Arbor.*

L-methionine has been shown to be involved in the biosynthesis of the PR8 strain of influenza virus. The propagation of this virus is inhibited by structural analogues of L-methionine. If poliomyelitis virus has this same requirement its growth should be affected by the presence of DL-ethionine, an inhibitor of L-methionine. The Lansing strain of poliomyelitis virus was grown in a tissue culture medium of minced human embryonic tissue in Hank's solution plus Simms ultrafiltrate. As determined by mouse inoculation, the tissue culture fluids between the 2nd and 11th days had maximum titers between 10^{-3} and 10^{-4}. When DL-ethionine in a concentration of 2.98 mg/ml. was added to the fluid a suppression in viral growth approximating 2 logs was observed. This suppression was almost complete when the quantity of inhibitor was doubled (5.86 mg/ml.). Subsequently when DL-ethionine was not incorporated in the fluid, virus propagation was resumed. This inhibitory effect is similar to that observed with influenza virus in tissue culture. Other structural analogues of normal metabolites are being tested by tissue culture technique which offers a convenient tool for biochemical studies of requirements for virus growth.

Tissue culture studies on liver cells of tuberculin sensitized animals in the presence of tuberculin (purified protein derivative). SONJA M. BUCKLEY AND JOHN J. BUCKLEY (introduced by FRANK MALTANER). *Div. of Exptl. Chemotherapy, Sloan-Kettering Inst. for Cancer Research, and Div. of Labs. and Research, New York State Dept. of Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

A difference between the anaphylactic type of hypersensitivity and the tuberculin type has been clearly demonstrated in tissue cultures of cells of mesenchymal origin, namely fibroblasts, polymorphonuclear leucocytes, lymphocytes, macrophages, and young marrow cells (RICH AND LEWIS. *Bull. Johns Hopkins Hosp.* 50: 115, 1932). Furthermore, it has been shown that epithelial hepatic cells from Arthus sensitized animals are unaffected by contact with the specific antigen in tissue culture, whether in the presence or absence of specific antibody. The purpose of the present experiments was to test *in vitro* the effect of purified protein derivative (PPD) (Parke, Davis and Company) of tubercle bacilli on epithelial hepatic cells from tuberculin sensitized animals. Washed liver cells of normal and allergic rabbits were exposed to a given concentration of PPD. It was found that cells of allergic animals are much more sensitive to the effects of PPD than those of normal animals. Cellular injury and necrosis of allergic liver cells was observed at measured amounts of PPD by which normal liver cells were unaffected. Combinations of cells with allergic or normal plasma seemed to indicate that a humoral factor is not involved in the allergic reaction. It could not be determined in these experiments whether complement plays a role in the allergic reaction as it is well known that all plasma contains complement.

A systematic study of certain viruses isolated in the tropics of Africa and South America. III. Physical properties. JOHN C. BUGHER (introduced by R. M. TAYLOR). *Labs. of International Health Div. of Rockefeller Fndn., New York City.*

Work in progress is reported on the biophysical

studies of these viruses with reference to: *a*) Precipitability with protamine sulfate; *b*) size as determined by ultrafiltration and centrifugation; *c*) shape of the virus particles as shown by electron microscopy; *d*) stability in desiccation.

Metabolism of antigenic I* proteins; effect of antigen and host alteration. S. C. BUKANTZ, F. J. DIXON, G. J. DAMMIN* AND D. W. TALMAGE.* *Washington Univ. School of Medicine, St. Louis, Mo.*

The mechanisms involved in the metabolism of antigens have been investigated: *1*) in normal rabbits with tracer labelled I* bovine serum albumin, I* bovine gamma globulin (I*-BGG), and with I*-BGG altered chemically by azo conjugation or iodination to saturation; and *2*) with I*-BGG injected into animals after whole body irradiation, active immunization, passive immunization, and in a specific anamnestic response. I*-BGG disappeared from the circulation in three phases (75% in 24 hours, 92% in 96 hours, and virtually 100% in 9 days) and was never retained in or concentrated by tissues. Complement fixing antibody appeared by the seventh day, was maximal at the 10th, and disappeared by the 20th day. By contrast, R-salt-azo-biphenyl-azo I*-BGG and P-amino benzoic acid-azo-I*-BGG disappeared from circulation precipitously (90% loss in 6 hours and 99% loss in 24 hours) and were retained in the RE macrophages of several organs, notably spleen, liver, and lymph nodes, with an approximate tissue half-life of 3–7 days. The iodine saturated antigen left the circulation as rapidly as azo antigens, with less tissue retention. Urine excretion of I^{131} containing protein breakdown products reflected the blood and tissue elimination of antigen. X-ray (300 r or more) did not effect the non-immune rate of elimination of antigen, although the immune response was suppressed. The intravenous injection of antibody to I*-BGG produced a prompt decrease in circulating antigen in proportion to the amount of antibody injected. Finally, in a specific anamnestic response, antigen disappearance during first two days was at the usual non-immune rates and a typical immune rate began at the third day.

Incorporation of C^{14} leucine by antibody proteins. NORMAN BULMAN* AND DAN H. CAMPBELL. *Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*

Kooyman and Campbell (*JACS* 70: 1293, 1948) have reported that rabbits given intraperitoneal injections of C^{14} carboxyl labelled leucine incorporated this leucine into the serum proteins. The data obtained in these preliminary experiments indicated that not only actively formed antibodies but also passively transferred antibodies acquired radioactivity. Subsequent studies using somewhat modified and more quantitative techniques have failed to confirm the latter observation. While actively formed antibodies incorporated the C^{14} leucine to a marked extent little or no activity was acquired by the passively transferred antibody which is in agreement with the earlier work of Heidelberger *et al.* (*JBC* 144: 555, 1942). Studies were also made on the uptake of the radioactivity by other plasma proteins and erythrocytes.

Role of activated blood protease in anaphylactic and anaphylactoid reactions. KENNETH L. BURDON, RUFUS K. GUTHRIE* AND DELORES F. RICK.* *Dept. of Microbiology, Baylor Univ. College of Medicine, Houston, Tex.*

An essential relationship has been discovered between the calcium content of blood and its antitryptic action. Complete removal of the protease inhibitor from whole blood, plasma, or serum, may be accomplished at will, merely by precipitation of all the calcium. Serum specimens may be fully decalcified and freed of all inhibitor in one hour, are diluted only 1:1, and remain unchanged in antibody content. The proteolytic activity of such sera has been titrated by a sensitive fibrinogenolytic test and also be a newly devised, simpler method using rennin as the substrate. Results are expressed in terms of proteolytic units equivalent to the action of a standard, stable solution of crystalline trypsin. Freshly decalcified serum shows a certain degree of proteolytic activity presumably due to the protease already in the active state at the time the blood sample was drawn. Addition of peptone *in vitro* to decalcified serum from normal individuals, or of antigen to treated sera from sensitized or immunized animals, results in immediate activation of more protease. Activation also occurs *in vivo* during acute anaphylactic or anaphylactoid (peptone) shock. The protease titers of samples collected at the height of the reaction are usually at least double those of the same animals just before the shock injection. Such proteolytic sera produce anaphylaxis-like symptoms and death on injection into normal animals. Surviving animals become relatively refractory not only to further injections of proteolytic serum but to specifically inducted anaphylaxis.

Development of antibody following transfer of cells taken from lymph nodes of sensitized or immunized animals. MERRILL W. CHASE. *Rockefeller Inst. for Med. Research, New York City.*

When guinea pigs are sensitized with drugs such as picryl chloride, the white cells will confer upon normal recipient guinea pigs the specific dermal sensitiveness of the donors. Moreover, when the donors are rendered highly sensitive by special methods (CHASE, M. W. *Federation Proc.*

9: 379, 1950) and a sufficient amount (0.5–0.9 ml.) of washed splenic cells or cells teased from lymph nodes is injected into the recipient *via* circulation and peritoneal cavity, it is found that the recipient can possess within 2 or 3 days both a specific anaphylactic hypersensitiveness and circulating antibodies. The two effects mediated by white cells appear to be separable, namely development of contact-type sensitivity to the simple drug and appearance of circulating antibody. The acquired antibody becomes detectable usually by the second or third day, but its concentration rises until the fifth or sixth day. Preformed antibody has not been found in significant amounts upon extracting the cells, and antibody has not appeared following the injection of killed cells. The mere injection of guinea pigs with o-chlorobenzoyl chloride suffices to render them suitable as cell donors 7-10 days later: lymph node cells have elicited the appearance of antibody in the recipient animals within one day and also a pronounced anaphylactic hypersensitiveness of the uterus as tested in the Dale technique with o-chlorobenzoyl casein. By analogous procedure, cells of the lymph nodes of rabbits injected with sheep erythrocytes have been injected into a normal rabbit with resulting appearance of hemolysin.

Typing of Coxsackie viruses. Sequence of development of complement fixing antigens. GUILLERMO CONTRERAS (introduced by JOSEPH L. MELNICK). *Section of Preventive Medicine, Yale Univ., New Haven, Conn.*

Previous work in this laboratory had indicated that in order to obtain complement fixing antigens from the low titer strains of the Coxsackie or C viruses, it was necessary to carry out a partial purification and concentration of the original suspension. In a search for a more satisfactory antigen, large infective doses (about 100,000 LD_{50}) of each of 3 antigenic types were inoculated into newborn mice, and groups of animals were sacrificed at daily intervals. Complement fixing antigens of maximum titer were obtained from the skinned torsos of mice sacrificed between 24 and 48 hours after inoculation. Soon after the peak titer was reached, the level of antigen began to descend rapidly. In contrast, the curve showing the incidence of paralysis among the animals used as source of antigens, ascended as the level of antigen decreased. With such antigens the complement fixation test has been applied to the typing of virus strains isolated in this laboratory from flies, sewage, and patients. Antiserum against unknown strains were prepared in adult mice using the combined action of tissue suspensions and Freund's adjuvants. With a known strain it was shown that even a dose as small as 1–3 LD_{50} was able to produce a specific antibody response. Usually antigens were prepared for the unknown strains and tested with standard sera. Over ten antigenic types are now recognized. The results are in agreement with those obtained by the neutralization test.

Intracellular distribution of radioiodinated antigens. CHARLES F. CRAMPTON* AND FELIX HAUROWITZ. *Dept. of Chemistry, Indiana Univ., Bloomington.*

It was recently reported that the bulk of the radioactivity deposited in the livers of rabbits injected with I^{131}-iodoprotein antigens is bound to the proteins of the mitochondrial fraction obtained by differential centrifugation and subsequent precipitation with trichloroacetic acid (*Science* 112: 300, 1950). An extension of these observations has revealed that the pattern of intracellular antigen distribution depends on the time elapsing between injection and sacrifice of the animal. If the radioactivity of the proteins of the whole liver homogenate (in cpm/mg.) is taken as 1.0, the activity of the microsomal proteins dropped from about 2.0 at 5 minutes after injection to 0.5 at 9 hours after injection. The corresponding relative radioactivity of the mitochondrial proteins increased from 1.7 (5 minutes) to 2.8 (9 hours). As yet we are unable to explain why the microsomes contain a considerable part of the antigen for only a short period following injection, whereas the mitochondria account for a large percentage of the antigen at all times investigated, particularly several hours after injection. Possibly the microsomes play a role in the intracellular transport of the antigen from the cell membrane to the mitochondria, or perhaps the microsomes after fixing the antigen rapidly undergo conversion into mitochondria. The pattern of intracellular distribution 24 hours following injection was not influenced by previous sensitization to the antigen. However, the amount of antigen administered seems to play a non-specific role in the distribution pattern. Large doses lead to an appreciably higher recovery of radioactivity in the supernatant fraction in the short term experiments.

Pulmonary toxicity of New Castle Disease virus (NDV) in mice. FRED M. DAVENPORT. *Dept. of Epidemiology and Virus Lab., School of Public Health, Univ. of Michigan, Ann Arbor.*

Although lung lesions and death in mice and hamsters regularly follow the intranasal inoculation of large doses of certain egg passaged lines of influenza virus, the yield of virus is comparatively low. It has been suggested that the lesions so produced result from a direct toxic action of the virus, rather than from viral multiplication. In order to study the pulmonary toxicity of virus suspensions, in the absence of viral multiplica-

tion, other virus-host systems were selected. Large doses of NDV inoculated intranasally produce lung lesions and death in mice. The lesions cannot be reproduced by serial passage nor can virus be recovered following this procedure. The lesions are prevented by homologous immune serum but not by normal or heterologous immune serum. The gross and microscopic appearance of these lesions have been studied. No toxicity was manifest following intracerebral inoculation of unconcentrated NDV suspensions. Heat inactivation of virus suspensions abolishes both the capacity to hemagglutinate chicken erythrocytes and to produce lung lesions. Vaccinated mice develop hemagglutination-inhibiting antibodies and antitoxic antibodies but are not protected when challenged with NDV intranasally. This unexpected result appears related to the intravascular location of the antibody and the rapid rate of adsorption of virus to cells. Immune serum instilled intranasally before virus inoculation prevents lesions, but fails to protect in instilled as early as 5 minutes after inoculation. The results of preliminary experiments with influenza virus in guinea pigs are similar.

Hemoantibodies in mice of inbred strains. ISRAEL DAVIDSOHN AND KURT STERN.* *Dept. of Pathology, Chicago Med. School, and Mount Sinai Med. Research Fndn., Chicago, Ill.*

Hemoantibodies in the serum of mice showed considerable differences depending on strain derivation. In 10 strains (C57 Black, C57 Brown, C57 Leaden, C3H, dba, A, Akm, B alb C, Marsh-albino, I) some or all of the following antibodies were studied: natural and immune agglutinins and hemolysins for sheep, chicken, and human erythrocytes. Natural antisheep agglutinins were found with greatest frequency and in highest titers in some sublines of C57 Blacks. Natural anti-chicken agglutinins were found in all animals of strains tested, with exception of C3H and Akm; the highest titers occurred in C57 Brown, C57 Leaden, A, and C57 Blacks. Natural agglutinins for human erythrocytes were rare, low-titered, or absent. Natural hemolysins for sheep and human erythrocytes were absent, and for chicken erythrocytes very rare. Immune agglutinins and hemolysins for sheep, chicken and human erythrocytes were highest in C57 Blacks as compared with C3H, dba, A, Akm. Absorption experiments demonstrated independence of antibodies for sheep and chicken erythrocytes. The natural antisheep agglutinin in mice is different from the Forssman and the infectious mononucleosis type. The findings raise a number of questions which form the basis for three lines of investigations pursued at present. Are the differences in natural hemoantibodies *1*) accompanied by antigenic differences in the tissues of the inbred strains; *2*) determined genetically? *3*) Are the differences in immune response related to other biologic properties of inbred strains? (Supported, in part, by a research grant from the National Cancer Institute, National Institute of Health, Public Health Service.)

Mechanism of PABA bacteriostasis: competition with a new vitamin, *p*-hydroxybenzoic acid. BERNARD D. DAVIS. *U. S. Public Health Service Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

Mutants of *Escherichia coli* blocked in the synthesis of shikimic acid, a precursor of aromatic compounds, grow only slowly on a mixture of tyrosine, phenylalanine, tryptophan, and *p*-aminobenzoic acid (PABA). Growth is accelerated by addition of either shikimic acid, an excess of PABA, or wild type culture filtrate. A fifth aromatic metabolite, Y was therefore postulated to be formed readily from shikimic acid, and less readily from PABA. Y was demonstrated in wild type filtrate by paper chromatography combined with mutant response. Its presence in traces in Whatman No. 1 paper led us to test many aromatic compounds; the only active one, *p*-hydroxybenzoic acid (POB), gives maximal acceleration at 0.01 μg/ml. and is chromatographically identical with Y. Since POB structurally resembles PABA, a known antirickettsial chemotherapeutic, antagonism between these compounds was investigated (cf. SNYDER AND DAVIS, this issue). PABA was found to slow growth of wild type *E. coli* at 200 to 1600 μg/ml.; POB in a 1/200 ratio completely overcomes this inhibition. 4,4′-Dihydroxydiphenyl sulfone also behaves as a competitive analogue of POB. Even an excess of either analogue produces only partial inhibition,—i.e. a partial POB requirement; similarly no available mutants have a complete POB requirement. In all cases this requirement becomes complete on addition of remarkably little L-aspartic acid (1 μg/ml.), without change in the POB/PABA competitive ratio; yet even at 3000 μg/ml. aspartic acid alone does not prevent growth.

Principles governing the practical application of complement-fixation tests. JOSÉ O. DE ALMEIDA,* ARTHUR M. SILVERSTEIN* AND FRANK MALTANER. *Div. of Labs. and Research, New York State Dept. of Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

In a study of the syphilitic system it has been found, as indicated previously in other systems, that complement fixation is an equilibrium phenomenon. The various relations which may be derived from this assumption to represent the interaction of complement with syphilitic antibody-cardiolipin complex were confirmed experimen-

tally. Utilizing these concepts, the authors have subjected to critical analysis the methods for the determination of titer of syphilitic sera. It has been possible to rationalize adequately many practices which were accepted on purely experimental grounds and to indicate the criteria to be used in selecting a method for evaluation of titer.

Radiosensitive and radioresistant phases in antibody production. F. J. DIXON, D. W. TALMAGE* AND S. C. BUKANTZ. *Washington Univ. School of Medicine, St. Louis, Mo.*

The radiosensitivity of the antibody response is well known, but the time relationships of radiation, antigen administration, and antibody suppression or response have not been completely explored. The following observations were made were made in rabbits using I^{131} labelled bovine gamma globulin as antigen. Antibody was determined by complement fixation, employing four 50% units. *1*) Antibody response to initial antigen exposure is completely suppressed by 300 r or more roentgens whole body radiation given 40 hours before injection of antigen. With 500 r, this suppression is evident for 6 weeks. *2*) However, no suppression of initial antibody response is noted following 500 r given simultaneously with antigen, and only partial antibody suppression follows 500 r given 2½ hours before the antigen. *3*) The specific anamnestic response, unlike the initial antibody response, is not prevented by 500 r 40 hours before the second injection of antigen. It is apparent that within a matter of hours after 300–500 r radiation, an animal losses a substance necessary for the production of antibody. However, since simultaneous radiation and antigen administration does not diminish antibody production in spite of the fact that the necessary radiosensitive substance can persist for only a matter of hours, it would appear that the radiosensitive portion of the antibody response lasts only a few hours. The remainder of the antibody response, during which the antibody protein is actually produced, lasts several weeks and can be carried on undiminished in spite of radiation. The complete radioresistance of the specific anamnestic response indicates a lasting orientation of antibody producing sites to the specific antigen, so that on second antigen administration the radiosensitive initiation phase of antibody production is unnecessary.

Bacterial activity of normal guinea pig serum for non-encapsulated strains of *Klebsiella pneumoniae*. JEAN A. DONNELLY* AND JAMES C. HUMPHRIES. *Dept. of Bacteriology, Univ. of Kentucky, Lexington.*

Non-encapsulated S form variants of *Klebsiella pneumoniae*, types A, B, and C have been found to be killed by fresh normal guinea pig serum. This bactericidal activity appears to be due to complement and natural antibody. Sera heated to 56°C. for 30 minutes showed no bactericidal activity. The activity was removed from sera without marked reduction in their hemolytic complement titers by absorption with suspensions of the susceptible bacteria in the cold (2°C.). However, mixtures of such complement active absorbed sera and heat inactivated sera were found to have regained their bactericidal activity. Sera were irreversibly inactivated by 70°C. for 30 minutes. The specificity of the bactericidal activity was tested by measuring the effect of heterologous absorption on the bactericidal titer for an S strain derived from a type A *K. pneumoniae* strain. Absorption by an S strain derived from a type B *K. pneumoniae* strain, and by a strain of *Corynebacterium xerosis* caused a marked reduction in bactericidal activity, whereas absorption with a type C derived S strain removed only approximately half of the activity and absorption with a strain of *Pseudomonas aeruginosa* removed none of the activity. The encapsulated M form parent strains were found to be resistant to the bactericidal activity of these normal sera. It would appear that the capsule acts as a physical barrier preventing contact between the somatic antigens and the bactericidal antibodies in these sera.

Hemagglutination by tularemia antisera and its inhibition by polysaccharide. RICHARD J. FEINBERG* AND GEORGE G. WRIGHT. *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*

Erythrocytes treated with polysaccharide from *Past. tularensis* become specifically agglutinable by various tularemia antisera. Optimum sensitization occurred when equal parts of 10% washed erythrocyte suspension and 0.005% polysaccharide were incubated 4 hours at 37°C. Thorough washing was then necessary to remove uncombined polysaccharide. Erythrocytes of human type O, A, and B, chicken, rabbit, guinea pig, mouse, sheep, and monkey were sensitized to the same degree under these conditions. In the presence of guinea pig complement sensitized sheep erythrocytes were lysed by tularemia antisera from man, horse, goat, rabbit, and rat; cells of other species listed above were agglutinated but did not show lysis. Polysaccharide was tightly bound to the erythrocytes, since repeated washing did not diminish their reactivity. Rabbits immunized with sensitized human erythrocytes developed antipolysaccharide antibody. Sensitization did not alter the natural agglutinability of erythrocytes by antisera, influenza virus, or botulium toxin, nor their susceptibility to lysis by lecithinase. Prior treatment of erythrocytes with lipase, periodate, or receptor destroying enzyme

of *V. cholerae* did not alter sensitization. Agglutination of the sensitized erythrocytes by antisera was strongly inhibited by free polysaccharide. Under appropriate conditions definite inhibition was noted in the presence of 0.001 gamma of polysaccharide.

Inhibition of vibrio El Tor hemolysin by some agents. OSCAR FELSENFELD. *Hektoen Inst., Chicago, Ill.*

The hemolysin of the El Tor vibrio is closely related to its lecithinase activity. Sulfadiazine, sulfamerazine, sulfamethazine, sulfanilamide and 12 amino acids failed to inhibit hemolysis, while lecithin, cephalin, phytin and BAL reduced the quantity of hemolysin produced *in vitro*. Streptomycin, neomycin, aureomycin and chloromycetin in amounts equivalent to one-tenth to one-half of their bacteriostatic doses significantly reduced the amount of hemolysin produced per vibrio. Penicillin, polymyxin B and D, subtilin, circulin, bacitracin and prodigiosin did not show such action. While formol and phenol in subinhibitory doses caused substantial changes in the agglutinability of the vibrios, no such effect was observed under the influence of antibiotics.

Failure of cortisone to affect rate of disappearance of antibody protein. EDWARD E. FISCHEL, HERBERT C. STOERK AND MOGENS BJØRNEBOE.* *Columbia Univ. College of Physicians and Surgeons, New York City, Merck Inst. for Therapeutic Research, Rahway, N. J., and Kommumhospitalet, Copenhagen, Denmark.*

It has been previously found that a depression of circulating antibody occurs in actively immunized animals within 6 hours to 3 days after ACTH or cortisone administration (*J. Immunol.* 61: 89, 1949; *J. Exper. Med.* 93: 37, 1951; *Am. J. Path.* 26: 708, 1950). This suggested that protein catabolism was accelerated by the hormones. To test this possibility, 4 rabbits were given equal amounts of rabbit antipneumococcus globulin solution intravenously. Two of the rabbits were treated with 10 mg. cortisone daily for 3 days before and 10 days following such passive immunization. After 20 minutes, 24 hours, 48 hours, and 4, 7, and 10 days, the 'labelled' globulin remaining in the circulation was determined by the quantitative agglutination method of Heidelberger and Kabat. The concentration of antibody remaining in the normal and cortisone-treated animals was similar, with a 'half-life' of about 6 days in all cases. The results suggest that cortisone does not increase the catabolism of the protein studied. The possibility is discussed that an inhibition of protein synthesis may explain the nitrogen loss accompanying cortisone administration.

Electrolyte changes during anaphylactic shock in mice. CHARLES L. FOX, JR.,* CARL T. NELSON AND ELIZABETH B. FREEMAN.* *Dept. of Bacteriology and Dermatology, Columbia Univ. College of Physicians and Surgeons, New York City.*

Electrolyte changes of considerable magnitude have been observed in the tissues of animals subjected to fatal shock by burns or trauma (*Am. J. Physiol.* 151: 155, 1947). Accordingly similar studies were conducted during anaphylactic shock. Mice were sensitized by intraperitoneal injections of 1 ml. of normal horse serum every second day for a total of 4 ml. Eleven to 15 days after the last injection, 1 ml. was rapidly administered intravenously. Fatal anaphylactic shock occurred in 50 of 71 sensitized mice. Normal mice given a similar intravenous injection showed no ill effects. The hind legs of these and shocked mice which died were amputated uniformly at the groin and analyzed for water, Cl, K and Na. The cation analyses were performed with our internal standard flame photometer (*Anal. Chem.* 23: 1951, in press). The results in mEq/liter of water were: shocked mice—K 108 ± 2.3, Na 106 ± 1.5, Cl 67 ± 1.3; controls, after receiving antigen—K 120 ± 3.2, Na 88 ± 4.9, Cl 52 ± 4.1. The hematocrit of pooled blood from shocked mice was 67% contrasted with 41% in the controls. Plasma K was markedly elevated and serum Na and Cl were increased but to a lesser degree. Urinary excretion of K, Na and Cl was subnormal for 24–48 hours after non-fatal shock. When sensitized mice were given 3.0 mg. of cortisone 18 hours prior to the challenging dose of antigen, all of 45 pre-treated mice survived. These data indicate that anaphylactic shock in mice is associated with hemoconcentration and profound changes in tissue electrolytes characterized by the movement of Na and Cl into and release of K from tissues. Cortisone inhibited the anaphylactic shock.

Indonesian Weil's disease in Puerto Rico and the United States. WILLIAM S. GOCHENOUR, JR., ROBERT H. YAGER, PSYCHE W. WETMORE,* LARUE B. EVANS,* ROBERT J. BYRNE,* AARON ALEXANDER* AND JOHN HIGHTOWER.* *Army Med. Service Graduate School, Washington, D. C., and Tropical Research Med. Lab., San Juan, Puerto Rico.*

During investigation of an epidemic of leptospirosis in Puerto Rico, leptospirae were isolated from 32 human patients, 10 rats, 2 mongooses and 1 dog by culture and/or animal inoculation of blood, cerebrospinal fluid, urine and kidney suspensions. Although not all strains isolated have yet been identified, one group is of sufficient importance to warrant this preliminary report. Strain identifications were made on the basis of

cross agglutination-lysis, antibody absorbtion and hamster protection tests (GOCHENOUR, YAGER, AND WETMORE, *Proc. Soc. Exper. Bio. & Med.* 74: 199, 1950) with known type strains. The leptospirae so far identified fall within 3 groups: *L. icterohaemorrhagiae* (isolated from cases of meningitis as well as of classical Weil's disease), *L. canicola*, and *L. battaviae*, the etiologic agent of Indonesian Weil's disease. Serologic evidence indicates that both rats and mongooses in Puerto Rico harbor *L. icterohaemorrhagiae* and *L. bataviae*. Leptospirae isolated from these animals are currently being definitely studied. Examination of a serum from a trapper in Louisiana, submitted by Dr. M. Raphael of the Veterans Administration Hospital, New Orleans, revealed the presence of agglutinating and lysing antibodies against *L. bataviae* in titer of 1:3200. These findings demonstrate the existence in North America of a human leptospirosis hitherto reported only from Asia, and emphasize necessity for more intensive critical investigation of the strains of leptospirae present in North America.

A theory for antigen-antibody reactions. RICHARD J. GOLDBERG (introduced by DAN H. CAMPBELL). *Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena, Cal.*

A statistical mechanical theory involving multivalent antigens, divalent antibodies, and univalent (blocking) antibodies has been developed on the assumption that all reaction sites are equally reactive. Three dimensional aggregates are formed which eventually become sufficiently large to precipitate. States of inhibition to states of precipitation are predicted in both antigen and antibody excess regions. Immunological differences between horse and rabbit antibodies are explained in the light of this theory. The Heidelberger equation for the amount of precipitate formed in the antibody excess region has been derived using this theory and its limitations have been studied. Ratios of antibody to antigen in the precipitate can be predicted for the range over which precipitation occurs for the two types of precipitation systems. Experimental values for these ratios are in agreement with theory. The theory indicates that the optimal proportions ratio is not necessarily the same for both of these systems. Blocking antibodies limit the range over which precipitation can occur. The relation of the antibody-antigen molecular ratio to the equivalent zone is discussed.

Significance of Schick test in adults. K. GOODNER AND A. M. DE ANDINO, JR. *Jefferson Med. College of Philadelphia, Philadelphia, Pa.*

Schick tests on succeeding classes of medical students reveal that the proportion of Schick-positives among young adults is gradually increasing. Under the usual interpretation this would be taken to mean that the proportion of non-immunes among the adult population has risen considerably during the last two decades. However with the minimal stimulus of the Schick test a large proportion of these apparent non-immunes was capable of developing Schick-negative or 'immune' status. The proportion of *potential* 'immunes' is therefore still high. The original Schick rates probably reflect only the lack of exposure to the organism or toxin. Single Schick test surveys of adult populations in regions where diphtheria is almost non-existent may have little or no value in determining the potentialities of the population with reference to diphtheria immunity. The practice of instituting immunization in adults on the basis of a single positive Schick test may well be questioned especially if one considers the nunber of severe reactions which follow the use of diphtheria toxoid in adults.

Transfer of cells from lymph nodes of rabbits following regional injection of antigens. SUSANNA HARRIS* AND T. N. HARRIS. *Children's Hospital of Philadelphia and the Univ. of Pennsylvania, Philadelphia.*

It has recently been observed by Chase that following repeated injections of antigenic material in guinea pigs cells could be teased from the regional lymph nodes and spleen which, when injected into fresh guinea pigs, conferred dermal hypersensitivity and gave rise to anaphylactogenic antibodies and precipitins. In the present study dysentery organisms were injected into the foot pads of rabbits and the popliteal lymph nodes were excised 4 days later. Cells were teased from these nodes and after repeated washing were injected intravenously or intraperitoneally into fresh rabbits. The latter showed homologous agglutinins on the first day thereafter, with gradually increasing titer until the 3rd or 4th day. A plateau was maintained at this level until the 7th or 9th day, after which there was a decline over the period of the next month. The transfer of cell suspensions which were frozen and thawed three times in a bath of dry ice and alcohol did not result in the appearance of antibodies in the serum of the recipient rabbit. Because of the effect of freezing and thawing and other treatments of the cells, and on the basis of quantitative considerations, it is felt that the antibody which appears in the serum of rabbits receiving these cells cannot be attributed to the passive transfer of preformed antibody nor to the carryover of antigen which could cause active immunization.

Effect of ACTH and cortisone on antibody. W. PAUL HAVENS, JR. AND JAMES M. SHAFFER (introduced by KENNETH GOODNER). *Dept. of Preventive Medicine, Jefferson Med. College, Philadelphia, Pa., and the Army Hepatic and Metabolic Center, Valley Forge General Hosp., Phoenixville, Pa.*

The effect of ACTH and cortisone on the amounts of circulating diphtheria antitoxin which appeared as an anamnestic response in *Schick-negative* patients at various intervals after the intramuscular inoculation of 50 Lf *purified* diphtheria toxoid was studied. The pattern of production of diphtheria antitoxin in control subjects showed quantitative variations, but qualitatively was characterized by an increase, detectable by the 3rd to 5th day, reaching a maximum between the 10th and 21st days, and declining thereafter. ACTH or cortisone was administered to 20 patients in different dosages and at variable times during the post-inoculation period. The amounts of circulating antibody found after the administration of these materials suggest that their use early (1st to 17th day) in the period of development of antibody, when the titer was *increasing*, and later (25th to 30th day), at a time when the titer was *declining*, apparently did not alter the pattern of response qualitatively as compared with the control subjects. A quantitative difference in intensity of response could not be determined from the data. It was of interest, however, that in 11 patients whose circulating diphtheria antitoxin was determined 4 or 8 hours after a single dose of 40 or 50 mg. of ACTH, a slight reduction was found. The possibility that this represented a 'blocking' or catabolic effect of ACTH is considered.

Teschen disease (viral encephalomyelitis of swine). DOROTHY M. HORSTMANN (introduced by JOHN R. PAUL). *Section of Preventive Medicine, Yale Univ., New Haven, Conn.*

Teschen disease of swine is a virus encephalomyelitis which, although its occurrence is unknown on the American continent, has been enzootic and epizootic in eastern Europe for at least 20 years. Since attention has been called to supposed similarities between it and poliomyelitis, studies were undertaken to attempt a classification of Teschen among the neurotropic viruses. No susceptible host other than the pig was found, although monkeys, newborn and young mice and hamsters, cotton rats, meadow voles, eggs and tissue culture were tried. The size of the virus by filtration through gradacol membranes was found to be between 10 and 15 mμ. Pathologically the disease is a diffuse encephalomyelitis with heaviest involvement of the cerebellum, thalamus, basal nuclei and spinal cord. Monkeys and rodents which had been previously inoculated with Teschen virus were as susceptible as controls to Lansing strains of poliomyelitis virus introduced intracerebrally. No interference could be demonstrated in mice between Teschen and Y-SK poliomyelitis, FA, or EEE viruses. Mice immunized against Teschen virus were as susceptible as controls to Y-SK and Lancing strains of poliomyelitis virus, Theiler's TO and FA strains of mouse encephalomyelitis, St. Louis, EEE, and Japanese B encephalitis viruses. Teschen virus appears therefore to be a distinct agent with a unique host range, a characteristic pathological picture, and with no antigenic relationships to any of the above viruses.

Limitation of alimentary virus carriage in chimpanzees immunized against poliomyelitis. HOWARD HOWE. *Poliomyelitis Lab., Johns Hopkins Univ., Baltimore, Md.*

In connection with a more extensive study, the frequency with which poliomyelitis virus could be recovered from the stools of chimpanzees has been investigated in 3 groups of animals—those receiving virus for the first time, and in immunized animals receiving homotypic and heterotypic viruses. Immunization was carried out by intramuscular vaccination with active and inactive virus, or by the induction of alimentary infection by oral inoculation. Stools which had been collected between the 7th and the 21st days following the test feeding of virus to the chimpanzees were assayed for the presence of virus by transnasal inoculation into pairs of rhesus monkeys. Of 48 stools collected from 12 previously uninoculated chimpanzees, 63% were found positive while of 36 stools from 9 immunized animals which escaped paralysis following homotypic challenge but nevertheless became carriers, 33% were positive ($P = .015$). The control animals eliminated virus for an average of 17 days while the immunized animals averaged 10 days. There was no demonstrable difference between vaccinated animals and those which had suffered previous alimentary infections. Immunized animals which had received homologous or homotypic oral challenges were also compared with those receiving heterotypic challenge. Of 40 animals receiving homotypic challenge 78% remained virus free, while but 33% of 18 animals were free of virus following heterotypic challenge ($P = .002$). The latter did not differ significantly from the controls. It is therefore demonstrated that immunization reduces the intensity and duration of homotypic infection but has no demonstrable effect upon heterotypic strains.

A soluble antigen from Herpes simplex virus giving specific skin reactions. ERNEST JAWETZ. *Div.*

of Bacteriology and Medicine, Univ. of California Med. School, San Francisco.

Allantoic and amniotic fluids from embryonated eggs infected with herpes simplex virus are suitable skin-testing antigens. This property in the past has been assumed to be due to the virus particles present in such fluids. However, virus particles sedimented from egg fluids and resuspended in saline no longer give positive skin tests in known reactors. The reacting substance remains in the supernatant fluid. Whole allantoic fluid was harvested from 12-day-old embryonated eggs infected with herpes virus on the 7th day of incubation. It contained $10^{6.7}$ infective units/ml. by titration on the chorioallantoic membrane. After inactivation with ultra-violet light, 0.1 ml. of the material injected intracutaneously gave an average reaction of 17.6 ± 1.2 mm. diameter in 31 subjects. The sediment obtained by centrifuging this fluid at 13,000 × *g* for 1 hour was made up to volume in buffered saline and contained $10^{6.0}$ infective units/ml. It gave skin reactions of a mean diameter of only 7.4 ± 0.97 mm. in 24 subjects. Although the supernatant fluid contained less than 0.5% of the original virus concentration it resulted in skin reactions of 18.5 ± 1.5 mm. mean diameter. The erythema, induration and time sequence of the delayed reaction was similar with whole allantoic fluid and with the supernatant obtained from 2 different egg-adapted strains of herpes virus. It appears, therefore, that the substance is a soluble antigen. It withstood heating at 56°C. for 2 hours (reactions measuring 22.8 ± 2.5 mm). It was present in only low concentration, because 10-fold dilution markedly reduced the size of the skin reaction (8.1 ± 1.6 mm). The soluble antigen is nontoxic to mice by the intracerebral and intravenous route, and can fix complement in the presence of antiherpes sera.

Variation in antiphage activity of the somatic antigen of Phase II *Sh. sonnei*. MARGERIS A. JESAITIS* AND WALTHER F. GOEBEL. *Rockefeller Inst. for Med. Research, New York City.*

The method of killing the microorganisms prior to their harvesting determines, to a considerable extent, the anti-phage activity of the chemically purified somatic antigen of Phase II *Sh. sonnei*. In addition, the serological and chemical properties of different preparations show some variation. Cells killed with 1% formalin yield an immunologically active lipocarbohydrate-protein complex which inhibits strongly the T_4 virus and to a lesser T_3 and T_7. When the bacilli are killed with 1% phenol, the serologically active component fails to inhibit materially any of the phages. Subsequent digestion with proteolytic enzymes yields a product with enhanced serological activity, and which now inhibits the T_3 and T_7 phages. It has but slight inhibitory action against the T_4 virus. The substance isolated from formal killed cells contains approximately 5% nitrogen and 3.2% phosphorus, whereas that obtained from phenol killed bacilli contains 12% nitrogen, and 1% phosphorus. After digestion, the latter yields a product which chemically, at least, is very similar to that obtained from formal killed bacilli. By dissociation with 90% phenol both antigens yield a lipocarbohydrate complex containing 3.1% nitrogen and 3.8% phosphorus. These substances still retain their antiphase activities. Analyses of the hydrolytic products reveal that they contain 30% phospholipid, 19% glucose, 9% glucosamine, 8% amino acids, 11% phosphoric acid, and 23% of an unidentified component.

A systematic study of certain viruses isolated in the tropics of Africa and South America. V. Histopathological lesions produced in chick embryos and laboratory mammals. HARALD N. JOHNSON (introduced by R. M. TAYLOR). *Labs. of the International Health Div., Rockefeller Fndn., New York City.*

As was noted in the section on cultivation of the viruses in chick embryos, specimens were taken for histological study in order to determine whether the multiplication of the virus was associated with abnormalities in growth or destruction of tissue cells and what types of tissues were affected. The type and location of lesions produced in white laboratory mice by infection with the viruses will be described. Hamsters are susceptible to infection with some of the viruses included in this study and the pathology of the disease produced in this host will be included.

Inhibition of Columbia SK virus hemagglutination by polio-convalescent human sera. CLAUS W. JUNGEBLUT AND BENI HORVATH.* *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.*

A total of 80 sera, obtained from patients during the acute or subacute stage of illness diagnosed as abortive or paralytic poliomyelitis, were examined for their ability to inhibit the hemagglutinating effect of Columbia SK virus on sheep red cells. The results were interpreted by comparing the action of these sera with the action of normal monkey serum on the hemagglutinating system. Thirteen human sera in this group were capable of inhibiting the hemagglutination reaction to a significant degree; another 15 sera gave doubtful results. The remaining 52 sera were clearly negative. A comparison with the mouse neutralizing test which was carried out with some of the positive and negative human sera showed no consistent agreement between the *in vitro* and *in vivo* method for antibody determination.

Effect of cortisone on experimental acute disseminated encephalomyelitis. ELVIN A. KABAT, ABNER WOLF AND ADA E. BEZER.* *Depts. of Neurology, Bacteriology and Pathology, College of Physicians and Surgeons, Columbia Univ., and the Neurological Inst., New York City.*

The effect of cortisone on the acute disseminated encephalomyelitis produced in rhesus monkeys by injection of homologous brain emulsions with the Freund adjuvants (paraffin oil, aquaphor and killed tubercle bacilli) was investigated. None of 6 monkeys given 40 mg. cortisone daily for 1 week prior to the administration of brain emulsion with adjuvants and for 10 weeks thereafter developed symptoms or showed pathological changes, while all of 6 control monkeys showed characteristic symptoms and pathology of acute disseminated encephalomyelitis. In a second experiment all of 6 controls were positive, while only 2 of 6 animals receiving 10 mg. and 2 of 6 given 5 mg. of cortisone 6 times weekly developed encephalomyelitis during a similar period. One of 3 animals given 17.5 mg. cortisone for 3 weeks and none of 3 given 17.5 mg. for 6 weeks showed pathological changes. Examination of the sites of inoculation of the brain emulsions showed marked inhibition of the granulomatous response to the tubercle bacilli in the cortisone-treated animals as compared to the controls. The data suggest, in accord with the findings of Ragan *et al.* for wound healing, that the mode of action of cortisone lies in its modification of the local granulomatous response previously established to be necessary for the production of the disseminated encephalomyelitis.

Universal serologic reactions of rabbits injected with tissue lipids. REUBEN L. KAHN AND HELENE PETRENCO.* *Univ. Hospital, Univ. of Mich., Ann Arbor.*

This study was undertaken to determine whether the injection of rabbits with tissue lipids affects precipitation in the universal serologic reaction with lipid antigen in these animals. Normal rabbits give varying degrees of precipitation in this reaction, while the same rabbit gives a relatively constant degree of precipitation. This finding suggests the presence of natural antibodies to lipids—of different concentrations in different rabbits and of approximately the same concentration in the same animal. With regard to the response of animals to injected lipids, it is generally assumed that lipids (haptens) are incapable of calling forth antibody production unless they are injected in combination with a protein. It was observed that rabbits injected with tissue lipids (present in Kahn antigen) without protein showed an increase in precipitation in their universal reactions over their normal precipitation levels. The increase was noted generally after 6 or more injections of lipids given at approximately weekly intervals. With the discontinuance of the injections precipitation was gradually reduced. It is believed that the natural antibodies to lipids present in normal rabbit serum are auto-antibodies formed in response to antigenic tissue lipids liberated in normal catabolism; also that the increase in precipitation in the injected rabbits is the result of increased auto-antibody production associated with an increase in catabolism and in liberation of antigenic lipids as a result of the injections.

Universal serologic reactions of rabbits injected with Freund's adjuvant. REUBEN L. KAHN, ALBERT H. WHEELER AND ELLA M. BRANDON.* *Univ. Hospital, Univ. of Mich., Ann Arbor.*

A combination of paraffin oil, lanolin-like substances and killed tubercle bacilli (Freund's adjuvant) has been found by different workers to augment specific antibody production to the injection of antigen. In this laboratory, it was primarily desired to find the extent to which precipitation in the universal serologic reaction with lipid antigen in rabbits might be affected by the injection of saline extracts of various tissues if Freund's adjuvant were also employed. It was found that, following such injections, precipitation in the universal reaction became markedly increased over the normal precipitation level shown by the animals. Then it was observed that Freund's adjuvant injected in rabbits without the tissue extracts also resulted in a marked increase in precipitation. When the ingredients of Freund's adjuvant were injected individually, it was found that the use of paraffin oil or killed tubercle bacilli resulted in increased precipitation, while the use of lanolin-like substances had little effect on precipitation. It is believed that the increase in precipitation in the universal reaction is associated with the tissue injury resulting from the injections of the paraffin oil or tubercle bacilli. This injury presumably augments the liberation of antigenic lipids and the resulting production of acquired auto-antibodies.

Studies in complement-fixation. II. Establishment of optimal concentrations of serum and cerebrospinal fluid for diagnosis. JOHN F. KENT AND HANS NEURATH. *Depts. of Bacteriology and Biochemistry, Duke Univ. School of Medicine, Durham, N. C.*

The effects upon complement (C′) of preliminary incubation with human serum or cerebrospinal fluid (CSF) were investigated; normal and syphilitic test fluids were examined, alone and with cardiolipin antigen, in comparative studies conducted with and without addition of Mg^{++} and Ca^{++} in concentrations optimal for immune hemol-

ysis. When divalent cation (DC) is not added, hemolysis by C′ is subject to opposing influences of test fluid, namely enhancement by endogenous DC and inhibition by a normal function characteristic of inactivated serum. Equilibrium between these influences is a function of test fluid concentration. With increasing concentrations of serum it shifts progressively from enhancement to inhibition of hemolysis and a neutral intermediate concentration may be established as optimal for diagnosis by complement-fixation. With the addition of DC, the hemolytic unit of C′ is considerably decreased, and complement-fixation tests employing a specified number of units exhibit increased sensitivity to antigen-antibody complex. Under the conditions, however, endogenous DC of test fluid cannot further enhance hemolysis and normal inhibitory properties of serum appear greatly increased. Unfortunately, when serum concentration is reduced to minimize inhibitory (anticomplementary) reactions, the sensitivity to fixation gained by introducing DC is sacrificed. In the case of CSF, however, inhibitory properties are normally minimal. Accordingly, the quantity introducible in complement-fixation tests, whether conducted with or without added DC, is restricted only by the volume allotted to test fluid, and the sensitivity afforded by added DC is fully realized.

A systematic study of certain viruses isolated in the tropics of Africa and South America. II. Cross-complement fixation tests. J. AUSTIN KERR (introduced by R. M. TAYLOR). *Labs. of the International Health Div., Rockefeller Fndn., New York City.*

The antigens were saline extracts of infected mouse brains from which the lipids had been previously extracted either by acetone and ether or by benzene. The antisera were prepared either in guinea pigs or in albino mice. Guinea pigs were immunized either with guinea pig or hamster brain material; mice were immunized with mouse brain material. The Kolmer technique was used, with overnight fixation in the cold room, and with a total volume of 0.6 ml. of reagents in each tube. In addition to the usual controls, all reagents were tested for reactivity to mouse encephalomyelitis before being considered satisfactory for use.

Fecal and serum antibody response to crystalline egg albumin. MARIAN ELLIOTT KOSHLAND. *Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.*

Recent studies (KOSHLAND AND BURROWS, *J. Immunol.* 65: 93, 1950) of the fecal and serum antibody to cholera vaccine indicated that fecal antibody represented the rate of antibody formation and that serum antibody represented an accumulation of antibody. The antibody response in guinea pigs to crystalline egg albumin was investigated to determine the effect of the nature of the antigen on the relationship between fecal and serum antibody. The method employed was the quantitative complement fixation procedure of Mayer *et al.* (*J. Immunol.* 59: 195, 1948). In a series of experiments in which a single intraperitoneal inoculation of egg albumin and adjuvant was used, relatively high serum titers were obtained, but no fecal antibody was detectable within the limits of experimental error (plus or minus 0.1 50% complement units). With the egg albumin-adjuvant antigen the ratio of fecal to serum antibody was less than one-twentieth of that observed in the cholera experiments. It was tentatively concluded that fecal antibody was not transported to the bowel in the bile or by migrating lymphocytes, but was produced by local cells, along the intestinal tract.

Immunochemical properties of antitoxin in normal and allergic individuals hyperimmunized with diphtheria toxoid. WILLIAM J. KUHNS, H. SHERWOOD LAWRENCE, AND A. M. PAPPENHEIMER, JR. *Dept. of Microbiology, New York Univ. College of Medicine, and the Bellevue Hospital Third Med. Service, New York City.*

Studies on sera from normal individuals hyperimmunized with purified diphtheria toxoid have demonstrated a close correlation between the *in vivo* antitoxin titers as determined by the rabbit intracutaneous technic and the *in vitro* titer as determined by quantitative precipitation. It was found that within experimental error 1 unit of human antitoxin is equivalent to 16 μg. specifically precipitable antitoxic γ-globulin. It has now been found that circulating antitoxins from several persons with marked allergic histories show highly significant discrepancies between *in vivo* and *in vitro* antitoxin titers. Despite the presence of 50–200 units of antitoxin per cc. as shown by rabbit skin test, the sera from a number of allergic patients contained only 4–8 μg. specifically precipitable antitoxic protein per unit. In the tubes showing maximal precipitation, 50–80% of the total antitoxin remained in the supernatant. In one patient whose serum contained 50 units/cc., no precipitating antitoxin whatever could be demonstrated. Passive transfer experiments using this serum indicated a high titer of skin sensitizing antitoxin when the site of passive transfer was tested 48 hours later with 0.01 μg. highly purified toxoid. These experiments indicate a possible identity between skin-sensitizing and non-precipitating antibody.

Chemical and immunological studies on purification of Vi antigen from *Escherichia coli* 5396/38.

MAURICE LANDY,* MARION WEBSTER,* MONROE E. FREEMAN* AND H. C. BATSON. *Biologic Products Div. and Dept. of Biochemistry, Army Med. Service Graduate School, Washington, D. C.*

Vi antigen, an important immunogenic component of *Salmonella typhosa*, occurs also in several other enteric species, *viz.*, *S. paratyphi C*, *S. ballerup*, and in a few coli strains. *Escherichia coli 5396/38* was selected for isolation of Vi antigen since quantitative studies made in these laboratories had indicated that it was the richest source of this antigen. The procedure for purification of Vi antigen was: *1*) extraction of acetone-dried organisms with 0.9% sodium chloride; *2*) fractional precipitation of the extract by ethanol at high salt concentrations; *3*) fractional precipitation of the most active fraction by ethanol at pH 3.4; *4*) acetic acid hydrolysis with subsequent precipitation of the active antigen by ethanol; and *5*) electrodialysis, which reduced the ash content from 15% to less than 1%. This procedure yielded a water soluble viscous acid which flocculated readily with albumin solutions near pH 4.0. This material had an acid number of 330. Tests for uronic acid were negative. The acid contained amino nitrogen, but qualitative tests for hexosamine, polypeptides and proteins were essentially negative. The maximum value for reducing sugars after hydrolysis was less than 4%. The purified Vi antigen possessed high mouse protective potency (ED_{50} = 0.001 μg.) against challenge with *S. typhosa*, but immunogenicity as measured by Vi antibody production in rabbits had been altered. The purified antigen exhibited a high degree of serological activity and specificity in collodion agglutination, hemagglutination, quantitative complement fixation and quantitative precipitin tests with typhoid, ballerup and coli Vi antisera. As determined by tests on the supernatants from quantitative precipitin tests with *E. coli 5396/38* antiserum, the purified Vi antigen appears to be immunologically homogeneous.

In vitro reactions of cercariae of *S. mansoni* with immune sera. CH'IEN LIU, FREDERIK B. BANG AND LAURENCE B. SENTERFIT (introduced by A. PAPPENHEIMER, JR.). *Dept. of Medicine, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

Three different types of reactions have been observed when living schistosome cercariae are placed in immune sera. They are: *1*) a precipitate formation; *2*) a membrane formation; and *3*) an agglutination of the cercariae when sufficient number are present. The precipitate reaction is clear only in fresh immune serum or heated immune serum reactivated by the addition of fresh normal guinea pig serum. On the other hand, no heat-labile factor is needed for the formation of the membrane or agglutination of cercariae. The development of the precipitate and agglutinin antibody occurs at the end of the incubation period and continues to be present in a high titer for many months. However, some monkeys and men who have been infected with schistosomiasis for several years and who are still actively infected as shown by positive stool examinations may give a negative test. Cross precipitate formation and agglutination of cercariae has been observed in the serum of monkeys infected with trichinella. There is no evidence of protection produced by one infection against the other, as shown by the trichina infected monkey which had a high agglutinin titer and which was still susceptible to a challenge of *Schistosoma mansoni* cercariae at a very small dose.

Chemoprophylactic effect of mercurial compound in suppressing intestinal carriage of Theiler's virus (TO) in mice. GERALD A. LOGRIPPO, DAVID P. EARLE, JR., IRVING GRAEF, AND ROBERT WARD (introduced by JULES FREUND). *Depts. of Medicine and Pediatrics, New York Univ.-Bellevue Med. Center, New York City.*

Unpublished studies in this laboratory have indicated that the feeding of certain mercurial compounds to normal mice was followed by a reduction in the concentration of Theiler's virus (TO) excreted in their feces. The present study was designed to determine whether or not mercury fed to TO virus-free mice would prevent the establishment of the intestinal carrier state during exposure to the virus. All TO virus-free mice became carriers within a week when exposed to mice excreting TO virus. However, the majority of TO virus-free mice, when alpha-mercapto-p-(2-thiazolylsulfamyl) acetanilide was added to their diet, failed to exhibit the virus in their feces after 1 week of exposure to virus-excreting mice. One week after discontinuing the drug, TO virus was found in the stools of most of the mice, even when the drug was continued for 7 days after removal from virus exposure.

Effect of cortisone on the pathogenesis of tuberculosis. MAX B. LURIE, PETER ZAPPASODI* AND ARTHUR M. DANNENBERG, JR.* *Henry Phipps Inst., Univ. of Pennsylvania, Philadelphia.*

Tuberculosis is associated with an hypertrophy of the adrenal cortex; the greater the hypertrophy, the greater the native resistance to the infection. Litter mates of inbred susceptible rabbits were divided into 2 groups. One received 2 mg. of cortisone acetate/kg. intramuscularly on alternate days throughout the study. The other served as controls. When the experimental animals were under the effects of cortisone, both groups were simultaneously exposed to the inhalation of viru-

lent human-type tubercle bacilli. It was found that, notwithstanding the greater *in vivo* phagocytic capacity afforded the reticulo-endothelial cells by cortisone, the number of inhaled bacilli initially arrested in the lung was no greater in the experimentals. Culturally, the bacilli were more numerous in the lungs of the cortisone rabbits. However, in the draining nodes of the controls the bacilli greatly outnumbered those of the experimentals. Five weeks after exposure the number of primary, visible tubercles in the lung was 4 times greater in the experimental rabbits. However, they were smaller, and their spread to the rest of the body was much less than in the controls. The lesions in the cortisone rabbits were caseous pneumonic foci with intraalveolar plugs of partially caseated cells swarming with inordinate numbers of bacilli. There was little perifocal inflammation. The lesions in the controls were tuberculous granulomas with well advanced, discrete caseous centers containing moderate numbers of bacilli and surrounded by widespread, perifocal inflammation. The permeability of the capillaries, as observed by Menkin, was reduced by cortisone. Nonspecific irritants as well as tuberculin induced much greater inflammation in the control rabbits. The role of reduced capillary permeability in this relation as well as in the more effective localization of the disease at the portal of entry that results from cortisone is considered. Withdrawal of cortisone caused dissemination of the disease and fatal issue.

Isolation of the pantothenate-synthesizing enzyme from *Escherichia coli*. WERNER K. MAAS (introduced by BERNARD D. DAVIS). *USPHS Tuberculosis Res. Lab., Cornell Univ. Med. College, New York City.*

Growth experiments with mutants have suggested the hypothesis that mutation affects a biosynthetic reaction by causing absence or alteration of the corresponding enzyme. This hypothesis can be tested more directly by comparison of the enzyme isolated from wild type and mutant. Biosynthesis of pantothenate in *E. coli* by peptidic coupling of β-alanine and pantoic acid seemed a promising test system: resting cells in the presence of precursors excrete pantothenate; this compound can be assayed accurately in low concentrations; several mutants blocked in this reaction have been isolated, including one requiring pantothenate at 37° but not at 25°. By extraction of acetone-dried cells of wild type *E. coli* with dilute pH 7 phosphate the coupling enzyme has been obtained in cell-free solution. Another enzyme system in the extract converts pantothenate slowly to a coenzyme A-like growth factor, active for *Acetobacter suboxidans* but not for pantothenate-requiring coli mutants. The acetone powder is stable in a vacuum desiccator at room temperature for at least 2 weeks. Pantothenate-synthesizing activity is removed by a 48-hour dialysis against dilute pH 7 phosphate at 5°, and is fully restored by addition of ATP. In this system pantoyl lactone is practically inactive as a substitute for pantoic acid. Characterization of the enzyme in extracts of wild type and mutants is in progress.

Serological changes during acute phase of anthrax infection. VIRGINIA G. MCGANN,* GEORGE G. WRIGHT, WINIFRED E. JONES* AND JANET B. SLEIN.* *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*

Study of serological changes in rabbits immunized with protective antigen preparations of varied potency and challenged with spores of *Bacillus anthracis* revealed that in nonimmune animals normal antihuman erythrocyte cold agglutinins, normal antisheep erythrocyte hemolysins, or normal T-agglutinins often decreased or disappeared entirely on the 3rd or 4th day after challenge. In the majority of animals decrease in antibody occurred abruptly within a few hours of death of the animal. Some partially immune animals that survived challenge showed a similar fall in antibody titers soon after challenge, but titers returned to normal within fourteen days. The abrupt change in serum antibody titers during acute infection indicated the presence of inhibitory substances. A number of acute phase serums, but not normal or convalescent serums from the same animals, were capable of inhibiting cold agglutination of human erythrocytes, hemolysis of sheep erythrocytes, or T-agglutination in normal and immune antiserums. High concentrations of acute phase serums inhibited precipitation of somatic polysaccharide and capsular polypeptide preparations of *B. anthracis* in hyperimmune antianthrax serums. In addition, many acute phase serums flocculated with hyperimmune antianthrax serums, whereas normal serums from the same animals showed no flocculation. With the exception of the substance affecting T-agglutination, all antigens that were demonstrated in acute phase serums by inhibition tests were also present in *in vitro* cultures of *B. anthracis*.

Epidemic of poliomyelitis characterized by dual infections with Coxsackie and poliomyelitis viruses. JOSEPH L. MELNICK. *Section of Preventive Medicine, Yale Univ., New Haven, Conn.*

Easton, Pennsylvania, experienced its first epidemic of poliomyelitis in 1949. This sharp outbreak was notable in the high ratio of paralytic to nonparalytic cases and the incidence of fatal cases. A group of patients who entered the hospital consecutively during the peak of the epidemic was

selected for detailed study. Of 36 patients (28 paralytic and 8 nonparalytic) studied for the presence of both poliomyelitis and Coxsackie (C) viruses in their stools, 20 were found to be excreting both viruses, 7 poliomyelitis virus alone, 6 C virus alone, and 3 were negative for both viruses. That these patients were actually infected with C virus and not merely carrying the agent in the intestinal tract is evidenced by the serological data. Of 26 patients excreting C virus, 23 responded with a rise in neutralizing antibodies to the Easton-2 strain or to the strain isolated from the patient. Of 15 patients from whom C virus could not be isolated, only 3 responded with antibody rises. It was found that with the rise of antibodies to C virus during the month after onset, there was a concomitant disappearance of C virus in the stools. In two paralytic patients studied, antibodies to both poliomyelitis and C viruses increased during the 4 weeks following the onset of illness.

Neutralizing antibodies against viruses of Coxsackie group in sera of wild rabbits. J. ANTHONY MORRIS (introduced by JOSEPH E. SMADEL). *Dept. of Virus and Rickettsial Diseases, Army Med. Service Graduate School, Washington, D. C.*

Sera from 17 of 57 wild cottontail rabbits (*Sylvilague floridanus*) trapped during 1948–1950 on the Patuxent Refuge, Laurel, Maryland, contained heat stable neutralizing substances against one or more strains of Group A Coxsackie virus. Sera that neutralized one strain of Group A virus were tested against one or more additional strains of Coxsackie virus and against encephalomyocarditis virus with the following results: 4 of the 17 sera neutralized only Group A, type 1 virus (strain T.T.), 4 neutralized only Group A, type 3 (strain O.L.), 6 neutralized only the Texas strain; 2 sera neutralized both Texas and T.T. strains, and 1 serum neutralized both Texas and O.L. strains. Ten sera which neutralized at least one strain of Group A virus were negative for neutralizing antibodies of Group B Coxsackie virus. In addition, 18 other sera failed to neutralize Group B virus. Similarly, 13 sera that neutralized at least one strain of Group A virus failed to neutralize encephalomyocarditis virus. Besides the 57 rabbits mentioned above, 4 rabbits were trapped twice during 1950, first in the summer and again in the fall. They were bled on each occasion and the paired sera were tested against the T.T., Texas, and P.O. virus strains. Paired serum samples from 2 of the 4 rabbits did not neutralize any of the three strains. Sera from 2 of the rabbits collected in June and July, respectively, failed to neutralize the Texas strain. Heated sera from the same rabbits collected in October neutralized between 2 and 3 logs of Texas virus. Serum collected in July from 1 of these 2 rabbits neutralized 2 logs of strain T.T. virus, while serum taken in October from the same rabbit did not neutralize significant quantities of virus. Sera from 117 wild birds and mammals belonging to 9 species were tested for neutralizing effect against one or more strains of Coxsackie virus. All were negative except for 1 of 2 chipmunks, 1 of 13 marmots and the only gray fox tested.

Immunological specificities and cross reaction in vascular beds of liver, kidney and lung. DAVID PRESSMAN AND BEILA SHERMAN.* *Sloan-Kettering Inst. for Cancer Research, New York City.*

The radioiodinated antibodies which localized in the kidneys, liver and lungs of donor rats injected with radioiodinated antibody serum were eluted with sodium hydroxide from the insoluble sediments obtained from the homogenates of the tissues of the donor rats, and these purified antibody preparations were tested for localization properties by injection into recipient rats. It was found that the purified radioantibodies from all 3 tissues localized to some extent in the kidney but the antibody isolated from each organ also showed preference for localization in the organ of initial localization. Liver-isolated anti-kidney antibody localized in the kidney and in the liver, but to a greater extent in the liver than did kidney-isolated antibody. Likewise, lung localized antibody localized to a greater extent in the lung, than kidney-isolated antibody. From the very rapid rates of localization of the antibodies it appears that the localization must take place on the vascular bed of the organ and the fractionation by heterologous organs of the antibodies prepared against one organ demonstrates serological specificities in the vascular beds of these organs. It is suggested that if certain perivascular diseases are caused by a process of autoimmunization, it is probably the localizing antibody which is the damaging agent and the organ which is attacked is determined by the specificity of the vascular bed of that organ.

Further observations on the JJ and 1233 influenza viruses. J. J. QUILLIGAN, JR., ELVA MINUSE* AND THOMAS FRANCIS, JR. *Dept. of Epidemiology and Virus Lab., School of Public Health, Univ. of Michigan, Ann Arbor.*

The 1233 virus, isolated in New York city in 1947, and the JJ virus, isolated in 1950 in Ann Arbor, Michigan, were both obtained during outbreaks of A prime influenza. They are practically identical in their biological behavior, growing well in the amniotic sac of chick embryos and adapting poorly to the allantoic sac as well as to other laboratory animals. They agglutinate chicken and human erythrocytes better at 6°C. than at 20°C. and do not agglutinate guinea pig erythrocytes.

Furthermore, the JJ virus shows fair agglutination of hamster and mouse cells and no agglutination of sheep or ferret cells. When the JJ virus is present in throat washings obtained with nutrient broth it does not withstand CO_2 ice freezing as well as storage at 6°C. In an attempt to demonstrate more clearly the clinical pattern resulting from infection with this virus, 6 adult women were inoculated with infected amniotic fluid and 5 others were given normal amniotic fluid. Close observation of these groups, which were maintained in isolation together, gave no indication of any illness although virus was isolated as late as 4 days after spray inoculation. However, one of the individuals who sprayed the infected amniotic fluid developed a mild illness starting 7 days after the spray was administered, and subsequently JJ virus was isolated. In retrospect it seems highly probable that this individual became infected at the time of the spraying procedure. The details of this volunteer experiment will be described.

Cultivation of poliomyelitis virus in monkey testicular tissue. WILLIAM F. SCHERER,* GEORGE BUTORAC* AND JEROME T. SYVERTON. *Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.*

The results of this study are presented in confirmation and in extension of the findings of Smith, Chambers and Evans (*Northwest Med.* 49: 368, 1950) which made it known that human testicular tissue supports the growth of human poliomyelitis virus. Four strains of poliomyelitis virus (murine-adapted Y-SK, murine-adapted Lansing, Mahoney and Minnesota-1948) are being propagated in tissue cultures by employing for the maintenance of cells derived from monkey testicular tissues a technique described earlier (*Proc. Soc. Exper. Biol. & Med.* 74: 509, 1950). A perforated cellophane membrane is employed for support of the cells. A gaseous mixture of 5% CO_2, 21% O_2, and 74% N is utilized daily to maintain a constant atmosphere in the flasks. The dilution factor at present for the Y-SK strain is 10^{45} based on tissue-fluid dilutions, or 10^{16} by computation for successive tissue replacements; that for the Lansing strain is 10^{13} on the basis of tissue replacements, or 10^{37} on the basis of fluid dilution. Since the original infectious titer of each virus was less than 10^3, the above dilution factors and the information that titrations carried out employing the supernatant fluids yielded a titer of 10^1 to 10^3, make it obvious that conclusive evidence for multiplication has been obtained. The results for the propagation of the Mahoney and Minnesota-1948 strain of human poliomyelitis virus in tissue culture will be reported at another time.

Effect of infection with influenza viruses on hemagglutination-inhibitors of tissues. R. WALTER SCHLESINGER AND HELEN V. KARR (introduced by JULES FREUND). *Div. of Infectious Diseases, Public Health Research Inst. of City of New York, Inc.*

Infection with influenza viruses is accompanied by progressive decrease in the amount of heat-stable virus hemagglutination inhibitors (VHI) extractable from tissues. This decrease is a sensitive indicator of viral activity and can be applied to organs, such as mouse brain, which do not lend themselves to measurements of receptor activity in terms of adsorptive capacity (*Soc. Am. Bact.* Abstracts of papers, 1949; *Proc. Soc. Exper. Biol. & Med.* 74: 541, 1950). Mouse brain VHI is more active against heated Lee than against A strains, and in this organ infection, even with A strains, leads to proportionately greater decrease in Lee-VHI. In the allantoic membrane of chick embryos, this relationship is reversed. VHI determinations on flask cultures containing minced allantoic membrane have been used in quantitative assays of the activity of receptor-destroying enzyme (RDE) of V. Cholerae. The method of VHI titration on infected tissue extracts has also been applied to a re-examination of the claim that influenza virus can infect cells whose receptors have been 'modified', i.e. rendered resistant to the enzymic action of virus or RDE, by appropriate treatment with meta-periodate (F. DE ST. GROTH. *Austr. J. Exper. Biol. & Med. Sc.* 27: 83, 1949). It was found that periodate under these conditions, and even in concentrations high enough to destroy part of the VHI, after a large proportion of VHI susceptible to progressive destruction by infecting virus.

Study of mixed precipitating systems by method of diffusion gradients: a comparison with quantitative precipitin technique. JOHN H. SCHWAB,* WILLIAM J. CROMARTIE* AND DENNIS W. WATSON. *Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.*

The method of diffusion gradients described by Elek (*Brit. J. Exper. Path.* 30: 484, 1949) when compared with the conventional quantitative precipitin technique, as applied by Cohn and his associates (*J. Immunol.* 61: 283, 1949), was found to be a valid, reproducible index of antigenic heterogeneity. The removal of a single component from a mixture of antigens resulted in the removal of a line of precipitate. However, the diffusion gradient technique as applied in precipitating-type systems demands a more precise control of the concentration of the reactants than has been reported for the flocculating system. In a system employing crystalline bovine albumin and anti-crystalline bovine albumin rabbit serum, the

sensitivity of the diffusion gradient technique with respect to the minimal amounts of antigen and antibody required to give a visible line of precipitate was 5γ antibody N and 0.5γ antigen N. The best procedure for detecting small quantities of antigenic impurities in a supposedly purified protein involved the hyperimmunization of rabbits with the protein and the use of a test antigen containing high concentrations of the suspected impurities. For example, crystalline bovine albumin (Armour) was found to be heterogeneous when assessed by anti-crystalline bovine albumin rabbit serum. Normal bovine serum served as the test antigen. These results were confirmed by the quantitative precipitin technique.

Production of cytotoxic nephritis in the dog by specific antiplacenta serum. BEATRICE CARRIER SEEGAL, MARGO WOLF HASSON*, EMILY NICHOLS LOEB* AND ABBIE I. KNOWLTON.* *Depts. of Bacteriology and Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.*

Rabbit antirat-placenta serum injected into rats produces both abortion and glomerulonephritis, the latter similar to that produced by rabbit antirat-kidney serum. Rabbit antidog-kidney serum produces acute and chronic glomerulonephritis in the dog. The present report describes the effect of rabbit antidog-placenta serum upon pregnant and non-pregnant female dogs. Rabbits were repeatedly immunized with dog placentas, using the adjuvant technique of Freund, and sera were collected frequently for 16 months. Four pregnant and 3 non-pregnant dogs received 2.5 cc. or 3 cc/kg. body weight of a pool of the antiplacenta sera, given intravenously on 3 successive days. The pregnant animals were injected about the 20th day of gestation. Two of these died 8 and 30 days later, having meanwhile developed oedema, hypertension, albuminuria, cylindruria, hematuria and nitrogen retention. Unlike similar experiments in the rat, neither dog aborted and the foetuses appeared normal. The macroscopic and microscopic findings in the kidneys were those of acute glomerulonephritis. The other 2 pregnant dogs, although they ran a similar but milder course and delivered healthy litters, continue to show persistent nephritis. Three non-pregnant dogs injected with antiplacenta serum also developed nephritis. One died in 9 days of acute nephritis, one is still under observation with signs of persistent renal damage, and one developed a mild nephritis which healed leaving no evidence at sacrifice 6 weeks after injection. Control dogs reacted as expected and the results will be discussed. (The work was aided by a Public Health Service grant.)

Response of mice to inoculation with certain avirulent bacteria after exposure to sublethal total body x-irradiation. I. L. SHECHMEISTER AND V. P. BOND (introduced by J. BRONFENBRENNER). *Dept. of Bacteriology, Univ. of California, Berkeley, and Biomedical Branch of Naval Radiological Defense Lab., San Francisco, Calif.*

Mice were exposed to sublethal dose of x-irradiation (350 r) and subsequently inoculated with live avirulent or dead organisms. When live bacteria were injected, the recovered organisms were either identical with the strain inoculated, or were apparently authogenous bacteria of intestinal origin. However, when heat-killed bacteria were introduced, the recovered organisms were of autogenous origin only. It is known that bacteremia, identified with normal bowel inhabitants, is often an integral part of the syndrome observed in animals dying from whole body irradiation (MILLER, HAMMON AND TOMPKINS. *Science* 111: 540, 1950). In this instance, however, bacteremia and death were induced in animals by sublethal doses of irradiation followed by inoculation with live avirulent or even dead organisms generically different from the bacteria normally present in the intestines of these animals.

Physical chemical nature of antigen-antibody complexes. S. J. SINGER* AND DAN H. CAMPBELL. *Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*

Preliminary unpublished results of the authors, and similar data recently reported by Becker and Munoz (paper presented at A.C.S. meeting, Chicago, Ill., September, 1950) had indicated that ultracentrifugal and electrophoretic investigations of antigen-antibody complexes formed between serum albumins and rabbit antibodies might yield valuable information concerning the nature of the immunologic reaction. In these studies, solutions of complexes of bovine serum albumin and rabbit antibody were prepared by dissolving in excess antigen specific precipitates formed by adding the antigen to pooled antisera. Ultracentrifuge experiments indicated the presence of two main resolvable classes of complexes. However, quantitative studies have been limited by two factors: *a*) the remoteness of the antigen-antibody ratios in such solutions from the equivalence zone; and *b*) the inability to determine precisely the values of these antigen-antibody ratios. The former condition was improved by making these solutions one-half saturated with neutralized $(NH_4)_2SO_4$, and redissolving the precipitate so formed in buffer. The resultant solution had a much lower antigen-antibody ratio. In order to remedy *condition b*, we prepared solutions of antigen-antibody complexes utilizing throughout as the antigen an iodinated bovine serum albumin

preparation containing an average of 5 iodine atoms per albumin molecule, together with rabbit anti-bovine serum albumin sera, in the manner previously described. Ultracentrifuge and electrophoresis experiments have been made with these solutions at a number of different, precisely-known, antigen-antibody ratios, total protein concentrations, and other experimental conditions.

Effect of ACTH on pneumonia induced with tuberculin in lungs of sensitized rabbits. DAVID T. SMITH AND O. M. REINMUTH.* *Dept. of Bacteriology, Duke Univ. School of Medicine, Durham, N. C.*

Old tuberculin was introduced in doses of 250, 125, and 100 mg. into the lungs of rabbits previously sensitized by a subcutaneous injection of Rl_V tubercle bacilli. One-half of the rabbits were given 10 mg. daily of ACTH in divided doses while the other half were kept as controls. A severe pneumonic reaction developed in the control animals which was obvious at 24 hours, reached its maximum at 4 days and disappeared by the 12th day. The reaction in the animals treated with ACTH varied from 10–40% of that observed in the controls. When ACTH treatment was withdrawn these animals developed within 3 days a fresh acute pneumonic reaction.

A systematic study of certain viruses isolated in the tropics of Africa and South America. I. Cross-neutralization tests. KENNETH C. SMITHBURN. *Labs. of International Health Div. of Rockefeller Fndn., New York City.*

During the latter 12 years of a long-term program of investigations on the epidemiology of yellow fever, a number of strains of virus were isolated from human beings, or from insects or wild animals infected in nature. As the agents were isolated they were all found to be distinct from yellow fever virus, and some were differentiated from other viruses. This and the following four papers are concerned with a systematic study of these agents by various techniques. All of the viruses are neurotropic in the sense that they induce fatal encephalitis in white mice on intracerebral inoculation. Ten well-known viruses which are either primarily neurotropic or have neurotropic potentialities were selected for comparison with them. Standard lots of each virus were prepared and assayed. Immune sera against them were prepared by subcutaneous inoculation of living virus into rhesus monkeys. A complete series of intracerebral or intraperitoneal cross-neutralization tests was then undertaken in order to determine which of the newly isolated viruses are distinct entities and whether any antigenic relationships among them or to the previously known viruses may be demonstrated. One of the African viruses was found, as already observed by others, to be identical with a virus previously isolated in the United States. The majority of the agents from both Africa and South America proved to be distinct from each other and from each of the 10 previously known viruses with which they were compared.

Reversal of rickettsiostatic effect of *p*-aminobenzoic acid by *p*-hydroxybenzoic acid. JOHN C. SNYDER AND BERNARD D. DAVIS. *Dept. of Microbiology, Harvard School of Public Health, and U. S. Public Health Service Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

The experiments herein reported were suggested by the observation that *p*-hydroxybenzoic acid (POB) competitively reverses the inhibitory effect of *p*-aminobenzoic acid (PABA) on the growth of wild type *Escherichia coli* (see DAVIS, B. D., this issue) and the fact that PABA in high concentrations (70 μg/ml.) inhibits the multiplication of pathogenic rickettsiae. Four experiments have been completed which show that POB reverses the static effect of PABA on rickettsiae under certain circumstances. Results are based on mortality curves, titrations of infectivity and toxicity of pooled yolk sac membranes in cotton rats and mice, and stained smears of homogenized suspensions of groups of several yolk sacs each, from two tests with the Breinl strain of epidemic typhus in chick embryos, one with murine typhus in white mice, and one with Rocky Mountain spotted fever chick embryos. For reversal of rickettsiostasis in chick embryos, the effective ratio POB/PABA (on the basis of the amounts inoculated into each egg as a single dose at zero time) appears to be in the range from 1/1 to 1/10.

Effect of autolytic changes on antigenic properties of kidney preparations. DOUGLAS H. SPRUNT AND ANNA DEAN DULANEY.* *Div. of Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis.*

In an earlier publication we reported the development of auto-antibodies to Staphylococcus-toxin-damaged rabbit kidney. Complement fixation tests demonstrated substantial titers for the *in vivo* damaged kidney antigens, and somewhat lower titers for normal kidney antigens. These antisera also proved nephrotoxic and in addition, produced anaphylactic-like reactions and skin necrosis in sensitized animals. Repetition and extension of these experiments have demonstrated the close correlation between *methods* and *period* of storage and antigenic properties. In all instances, the kidneys were removed aseptically from the rabbit immediately after the kidneys were perfused with cold physiological saline and ground in a refrigerated blender. The tissue sus-

pensions were then divided; one part being frozen immediately and stored at −20°C., the other portion being stored in the refrigerator at 4°C. The Sorensen formol titration test for free amino acids was used to demonstrate tissue breakdown. After storage in the ice box at 4°C. for periods of from 3–4 weeks, some of the rabbit kidneys stimulated the production of antibodies when injected into rabbits. These antibodies could be demonstrated by the Complement fixation test. The portion of the kidney which was stored at −20°C. did not produce antibodies. We have also found that the content of free amino acids in the preparation kept at 4°C. is greater than that at −20°C. These determinations were performed by the Sorenson formol test. From these experiments, we are suggesting that autolytic changes at ordinary ice box temperature is a factor in making tissues antigenic.

Role of regional lymph node in antitoxin formation following local injection of diphtherial toxoid. A. B. STAVITSKY. *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

The evidence which is presented implicates the popliteal lymph node in the production of antitoxin in rabbits following injection of a purified, alum-precipitated diphtherial toxoid into the corresponding foot pad or into this node. Three weeks after the primary injection of toxoid the lymph node was removed. Following this procedure about half the animals showed a marked decrease in the antitoxin content of their serums. One week after removal of the node the toxoid was injected into the foot pad. All animals developed a secondary rise in serum antitoxin following this 'booster' injection. However, the rise was very small compared to that in the controls which were similarly treated except for removal of the node. In general, after reaching its peak the antitoxin content of the serum dropped more rapidly in the rabbits from which the nodes were removed than in the controls. These effects on the primary and secondary antitoxin responses were most striking and consistent in animals in which the toxoid was inoculated directly into the lymph node. The occurrence of a secondary rise of antitoxin in animals from whom the node was extirpated indicates that organs other than the local node were involved in the production of antitoxin after local injection. However, the disparity between antitoxin levels in these animals and the controls suggests that the greater amount of antitoxin in locally injected animals was synthesized locally rather than systemically.

Effect of injection of O.T. on circulating antibodies in guinea pigs infected with tubercle bacilli. W. EMANUEL SUTER AND STEPHEN V. BOYDEN (introduced by R. J. DUBOS). *Rockefeller Inst. for Med. Research, New York City.*

Circulating antibodies against tuberculous antigens in rabbits infected with tubercle bacilli and in man suffering from clinical tuberculosis can be revealed by the hemagglutination test. Using sheep red cells sensitized with Lederle O.T., it is rarely possible to detect antibodies in guinea pigs infected with virulent, attenuated or avirulent strains unless large amounts of culture have been injected. However, intradermal administration of O.T. stimulates the production of serum antibodies in animals previously infected with BCG and other strains. In about one-third of the animals the injection of 0.1 ml. O.T. 1:1000 elicits the immediate appearance of transitory antibodies giving a typical pattern of sedimentation of the sensitized red cells. Agglutinating antibodies appear 2–7 days after stimulation and disappear within 15–30 days, depending upon the height of the initial titer. Tenfold increase of the amount of O.T. enhances the effect as does the injection by the intracardial route. There seems to be no correlation between intensity of tuberculin skin reaction and titer of antibodies after stimulation. The stimulating effect of O.T. is detectable in some animals within a few days after infection with BCG and is maximal 3–8 weeks later. Furthermore, the effect of O.T. is conditioned by the dose of BCG injected.

Role of spleen in hemolysin production after multiple antigen injections. WILLIAM H. TALIAFERRO AND LUCY GRAVES TALIAFERRO.* *Univ. of Chicago, Chicago, Ill.*

Previous work (*J. Infect. Dis.* 87: 37, 1950) on rabbit antisheep hemolysins, using 50% hemolytic units determined photometrically, indicated that, after a single intravenous antigen injection, the spleen forms most (mean 80%) of the antibody during the initial rise and then abruptly stops forming it. Splenic antibody then disappears at a constant logarithmic rate until a level is reached which represents the continuing antibody production of nonsplenic sources. In the present work it was found that peak hemolysin titers are on the average 3–5 times higher in multiply injected intact rabbits than in singly injected ones. The comparative role of splenic and nonsplenic antibody sources is, however, reversed. Thus, peak titers in splenectomized multiply injected rabbits, instead of being less, are 2–3 times higher than in similarly injected intact ones. This result was obtained with intermediate and large amounts of red cells. The intermediate amount of involved 1.25 ml. 1% sheep cells/kg. rabbit given 10 times during one month, and the large amount involved 1 ml. 10% sheep cells/kg. rabbit given 36 times during 2.5 months. Two possibilities suggest them-

selves to explain the reversal of the relative importance of splenic and nonsplenic sources for antibody: *1*) The mechanism which inhibits the activity of the spleen also affects nonsplenic sources to prevent maximal antibody-formation in multiply injected intact rabbits. *2*) In multiply injected intact rabbits, the spleen continues to remove antigen from the blood before it reaches nonsplenic sites, but is itself inhibited.

Antigen elimination as an index of immune response. D. W. TALMAGE,* F. J. DIXON, S. C. BUKANTZ AND G. J. DAMMIN.* *Washington Univ. School of Medicine, St. Louis, Mo.*

The rate at which I^{131} tagged bovine gamma globulin disappears from the blood of a rabbit has been found to be a sensitive indicator of the development of an immune response. Several experimental observations support this contention. *1*) A rapid elimination of antigen is found in the absence of circulating antibody when small amounts of x-ray are administered to the animal before antigen is given or when extremely small amounts of antigen are used. However, upon reinjection, these animals show a specific anamnestic response similar to that given by animals with previously detectable antibody. *2*) In normal control rabbits, the appearance of specific antibody coincides with the disappearance of antigen. *3*) In rabbits given 500 r whole body x-radiation, the antigen disappearance rate remains at the nonimmune level and no antibody is formed. *4*) When injected into a highly sensitized animal, the antigen leaves the blood at a rapid rate, beginning at the moment of injection. *5*) Injection of antibody into a rabbit with circulating antigen reproduces the rapid fall in blood antigen level which we interpret as an immune response. It seems likely that in the presence of circulating antigen the first antibody formed combines with the antigen, causing an increase in the rate of antigen elimination, which is noted several days before excess antibody becomes detectable in the serum. With an uninhibited antibody response, antibody continues to be produced after antigen disappears and is then detectable in the blood. However, in the event of limited antibody production in an immune response, there might never be any circulating antibody.

Factors influencing concentration and purification of new strains of influenza virus. A. R. TAYLOR AND I. WM. MCLEAN, JR. *Research Labs., Parke, Davis and Company, Detroit, Mich.*

Each year the number of new influenza virus strains or variants appears to be increasing. In general, the yields observed on cultivation of these new strains in the fluids of the developing chick embryo, as indicated by infectivity and hemagglutination measurements, are not at all comparable to those obtained with the classical PR8 and Lee strains. In this laboratory, comparative studies are being undertaken with several strains of current interest in order to obtain purified concentrates for a more detailed study of biological, chemical and physical properties and their interrelations. When the centrifugation techniques used successfully for the purification of PR8 and Lee viruses are applied to some of these more recently isolated strains it has proved difficult to obtain a reasonable degree of purification and the recovery of virus is low. Inhibitor substances and/or associated aggregation phenomena encountered in the freshly harvested infectious fluids, and in the concentrates derived from them, can be controlled by means of suitable buffer systems and by the use of certain specific agents. For example the Cuppett strain, now incorporated in the official influenza virus vaccine, was found to be far more amenable to centrifugal 'handling' in the presence of citrate ion. Use of this ion in the procedure resulted routinely in apparent increases in yield of the order of 50–100%. Marked differences have been observed in the sedimentation characteristics of the various strains studied, dependent on type and pH of the media.

A systematic study of certain viruses isolated in the tropics of Africa and South America. IV. Cultivation and behavior in chick embryos. R. M. TAYLOR. *Labs. of International Health Div. of Rockefeller Fndn., New York City.*

The first objective was to obtain satisfactory growth of each virus strain in serial passage in chick embryos. With most of the viruses this was readily accomplished by inoculation into the yolk or amniotic sacs. With some, intracerebral inoculation was required. After the virus had been established, separate groups of embryos were inoculated with a determined amount of the virus into the yolk, amniotic, and allantoic sacs and upon the chorioallantoic membrane. At varying intervals following inoculations three or more embryos were harvested and the virus content of different tissues and/or extra-embryonic fluids was determined by titration in mice. At the same time three additional embryos were preserved for histopathological study. Also, by inoculating graded tenfold dilutions the mortality as well as the infectivity of each virus for chick embryos was determined.

Protection of mice against the vaccinia virus by the administration of phenoxythiouracils. R. L. THOMPSON, S. A. MINTON, JR.,* ELVIRA A. FALCO* AND G. H. HITCHINGS.* *Dept. of Microbiology, Indiana Univ. Med. Center, Indianapolis, and Wellcome Research Labs., Tuckahoe, N. Y.*

Certain 5-phenoxythiouracils, although only moderately active inhibitors of vaccinia virus in

an *in vitro* screening test (*J. Immunol.* 55: 345, 1947) have shown the ability to protect mice against the virus. The degree of protection is significant with IHD strain inoculated either intracerebrally or intranasally and with the WR strain inoculated intracerebrally. The degree of protection may vary with the season of the year, and is not closely correlated with the size of the inoculum. On the average, less virus has been demonstrated in the brains of sacrificed treated mice than in comparable tissues from control animals. The chemotherapeutic agents may be administered intraperitoneally or orally, maximum effectiveness being achieved with 0.75% in a casein sucrose diet. 5-(2′,4′-Dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine appears to be the most active substance, but is closely rivaled by the 2,4-dibromo and the 4-bromo and 4-chloro derivatives. Compounds with electron donor substituents in the benzene nucleus in general are inactive. The group of antiviral agents is unusual in the absence or near absence of antibacterial effects.

Complement-fixation test for trichinosis. Victor N. Tompkins (introduced by J. Maltaner). *Div. of Labs. and Research, New York State Dept. of Health, Albany.*

Complement-fixation test for trichinosis using the coctostable alkaline-saline extract of dried larvae (Witebsky) was investigated using quantitative methods. The importance of multiple antigen dose, serum diluted with salt solution, and a small complement dose could be shown in achieving desirable sensitivity. The test was highly specific.

Action of high intensity electrons on microorganisms. I. Inactivation of bacteria and viruses. Frederick B. Traub,* Wolfgang Huber,* Arno Brasch* and Ulrich Friedemann.[1] *Dept. of Bacteriology, Jewish Hosp. of Brooklyn, and Research Labs., Electronized Chemicals Corp., Brooklyn, N. Y.*

High energy electron bursts, obtained from a 3 MeV Capacitron were employed in this investigation. The sterilization doses of a number of nonsporulating and sporulating bacteria and fungi, as well as for animal and bacterial viruses, were determined and found to vary from 150,000 rep (roentgen-equivalent-physical) for the most susceptible bacterium to 1,900,000 rep for the most resistant virus. It could be shown, that, with the exception of spores, the decrease of the size of the organisms required an increase in absorbed energy, in order to achieve complete inactivation. Inactivation curves were determined at various temperatures for E. coli, M. leisodeikticus, B. subtilis spores, rabies virus, and murine encephalomyelitis virus S. K. strain. It was found that the effect of a given dose of irradiation was the same, within the limits of error, at 10°C. and at −35°C. The identical radiation sensitivity of these microorganisms at different temperatures is surprising and in sharp contrast to the pronounced temperature coefficient for a given dose of radiation shown by such tissue components as enzymes, vitamins, plasma-proteins, complex carbohydrates and nucleic acids. There, 63% inactivation at −35°C. requires about 10 times the dose of radiation necessary at 10°C. The results, when evaluated in terms of the target theory of radiation-inactivation, were found to be in agreement with a 'single hit' mechanism of inactivation.

[1] Deceased.

Anaphylactic arthritis in guinea pigs and its hormonal control. G. Ungar, E. Damgaard and H. G. Weinstein (introduced by A. F. Coburn). *Rheumatic Fever Research Inst., Northwestern Univ., Chicago, Ill.*

Passive Arthus phenomenon was produced in guinea pigs by intracardial injection of anti-egg albumin rabbit serum followed by the introduction of egg albumin into an ankle joint. The arthritis thus induced was followed by recording the diameter changes of the antigen-injected joint compared with changes in the opposite joint which had been injected with an equal amount of saline. Swelling of the joint was found to be proportional to the log of antibody N injected. The swelling of the joint was reduced by treatment of the animals with pituitary corticotrophic hormone or cortisone. Cortisone was tested in splenectomized animals and was found to be without effect on this arthritis. A substance produced by the spleen (splenin A) also inhibited the swelling of the joint. The swelling could be increased beyond the control levels by treatment with desoxycorticosterone acetate, thyroid-stimulating hormone, large dosages of thyroxine and another substance produced by spleen and bone marrow (splenin B). Among other substances which decreased the intensity of this arthritis were Na salicylate, heparin, and certain anti-histaminics. An attempt will be made to explain the mechanism by which the pituitary, adrenal cortex, thyroid, and spleen may control certain inflammatory processes involved in local anaphylaxis.

Properties of a pancreatic polysaccharide. Ethel Verney* and Aaron H. Stock. *Dept. of Pathology and Bacteriology, Children's Hosp. of Pittsburgh, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

In a previous paper by one of us (*J. Bact.* 38: 511, 1939) dealing in part with possible sources of polysaccharides in certain peptones, the isolation

and chemical analysis of a polysaccharide in pancreatic products was reported. Other reports bear on this subject, viz., Mandel and Levene discovered mucoitin sulfuric acid in pancreas; Schiff and Weiler found commercial trypsin had blood group A activity; and Bray, Henry and Stacey isolated from pancreas and pancreatin a polysaccharide with blood group A activity. Although there have been studies on tissue polysaccharides from other sources, the data on pancreatic polysaccharide is incomplete. This paper deals with preparations of polysaccharides from whole pancreas or pancreatic products and gives a comparative analysis, including blood group activity, reducing substance, methylpentose, glucosamine, N, S and P. Characterization was attempted by ultracentrifugation and electrophoretic analysis. Since virulence enhancement has been reported as a property of samples of blood group A gastric polysaccharide, virulence enhancement of group A hemolytic streptococci with pancreatic polysaccharides was tried. Our object was not only to better define the polysaccharide from pancreas but to determine any chemical or biological differences which would differentiate it from gastric polysaccharide in biological products of unknown origin, as peptones. Some of our preparations of pancreatic polysaccharide lacked blood group A activity and had no blood group O or B activity. Blood group activity should not be the sole criterion for presence of polysaccharides in pancreatic products but should be supplemented by isolation and chemical analysis.

Path of biosynthesis of proline. HENRY J. VOGEL* AND BERNARD D. DAVIS. *U. S. Public Health Service Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

Of three proline-requiring *Escherichia coli* mutants isolated by the penicillin method, one (55-1) excretes a precursor (I); the second (55-25) responds to proline or I; while the third (22-64) responds to proline, I, or glutamic acid. In the conversion of glutamic acid to proline, cyclization could conceivably occur at the carboxyl, aldehyde, or alcohol level of oxidation. Since pyrrolidone-α-carboxylic acid and α-amino-δ-hydroxyvaleric acid are inactive for all the mutants, glutamic semialdehyde (γ-amino-γ-carboxybutyraldehyde), a probable catabolic product of proline (TAGGART AND KRAKAUR. *J. Biol. Chem.* 177: 641, 1949), has now been synthesized. The semialdehyde, obtained in high yield by refluxing γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde in 6N HCl, is essentially as active on a molar basis as DL-proline for 55-25, is inactive for 55-1, and is indistinguishable from I by paper chromatography combined with mutant response. Chemical properties of the synthetic product indicate that the initially produced semialdehyde, like other γ-aminoaldehydes, spontaneously forms an intramolecular aldehyde-ammonia or other cyclized structure in aqueous solution at physiological pH. It also is reducible to proline *in vitro*. It is concluded that the biosynthesis of proline includes enzymatic reduction of glutamic acid to glutamic semialdehyde followed by spontaneous cyclization and further enzymatic reduction. Glutamic semialdehyde cannot be the immediate precursor of ornithine in *E. coli*, as it does not support growth of any ornithine auxotroph, including one that responds to an unknown precursor accumulated by another. This ornithine precursor in turn is inactive for all three proline auxotrophs.

Significance of complement in tuberculin cytolysis. BYRON H. WAKSMAN. *Massachusetts General Hosp., Boston.*

It is well established that under appropriate conditions macrophages and fibroblasts from tuberculous animals fail to grow or migrate in tissue culture in the presence of tuberculin. The role of complement (C′) in this phenomenon is unknown. Favour finds C′ necessary for *in vitro* cytolysis of polymorphonuclear leukocytes and lymphocytes of tuberculous individuals. Tissue cultures of spleen and lymph node were prepared from guinea pigs with progressive tuberculosis and moderate to marked skin sensitivity to tuberculin as well as from normal controls. The inhibitory effect of tuberculin on proliferation and migration of macrophages was as clearly demonstrable in tubes containing no C′ as in tubes containing guinea pig C′. This inhibitory effect did not involve fibroblasts in a clearcut manner.

Mechanism of liquefaction of tubercles. CHARLES WEISS, FRANK SINGER* AND M. L. BOYAR-MANSTEIN.* *Div. of Microbiology, Labs. of Jewish Hosp., Philadelphia, Pa.*

Tuberculosis was produced in the lungs of unselected, albino rabbits by means of the Wells apparatus for quantitative air-borne droplet nuclei infection. The animals were killed at varying intervals after infection, thus permitting observations during successive stages in the development of discrete tubercles. Correlated chemical and histologic studies revealed evidence of greatly increased enzymatic activity (BA-amidase and LA-peptidase) in the inflammatory zones of these tubercles. The areas of lung intermediate between primary tubercles, which contained only microscopic areas of cellular infiltration, showed only a moderate increase in the rate of enzymatic hydrolysis. In the centers of the tubercles where there was gross evidence of caseation, the rate of substrate cleavage was markedly decreased. The

values for the rate of hydrolysis of LA became very low and those for BAA reached zero in areas where macroscopic softening had developed. It appears, therefore, that the rate of proteinase action is proportional to the intensity of cellular infiltration. In order to throw additional light on the intimate mechanism of cellular breakdown during caseation and softening of tubercles, various portions of the latter were homogenized and a comparison was made of their capacity to depolymerize DNA. Results with viscosimetric and colorimetric methods will be presented and their immunologic significance discussed. (Aided by a grant from the National Tuberculosis Association.)

The half-life of the antibody globulin molecule. ALEXANDER S. WIENER. *Serological Lab. of Office of Chief Med. Examiner of New York City, and Blood Transfusion Div. of Jewish Hosp. of Brooklyn, N. Y.*

The fate of Rh antibodies passively acquired by infants from their sensitized mothers was determined. The titers at various times after birth were substituted in the formula: $T_n = T_o e^{-kn}$, where T_o is the antibody titer at birth, T_n is the antibody titer on the n_{th} day, and $k = \frac{0.6932}{\text{half-life in days}}$. To allow for body growth, the right side of the equation is multiplied by $\frac{W_o}{W_n}$, where W_o is the birth weight, and W_n the weight on the n_{th} day. In one Rh-negative baby, the antibody titer at birth was 250 units, on the 93rd day 20 units, and on the 176th day 2 units, indicating a half-life of approximately 30 days. In a second Rh-negative baby with an initial titer of 20 units, the same calculated half-life was obtained. In a third case a mildly sensitized Rh-negative mother gave birth to an Rh-positive baby with subclinical erythroblastosis. The free Rh antibodies were eliminated from this baby's serum at the same rate as in the Rh-negative babies. Studies on alpha and beta antibodies passively acquired by babies from their mothers showed them to have the same half-life as Rh antibodies. Analysis of similar studies previously published showed that the half-life of 30 days was also valid for other antibodies such as antibacterial and antitoxic diphtheria antibodies. Furthermore, application of the formula, corrected for weight gain, in guinea-pigs and sheep, gives approximately the same half-life for antibodies in these species.

Metabolic studies of rickettsiae: pathway of glutamate oxidation in suspensions of *Rickettsia mooseri*. CHARLES L. WISSEMAN, JR.,* FRED E. HAHN,* ELIZABETH B. JACKSON* AND JOSEPH E. SMADEL. *Dept. of Virus and Rickettsial Diseases, Army Med. Service Graduate School, Washington, D. C.*

Studies of the biology of rickettsiae and the nature of their parasitic dependence upon host cells require knowledge of the independent metabolic capacities of these organisms. The oxidation of glutamate by rickettsiae reported by Bovarnick *et al.* (*J. Exper. Med.* 89: 561, 1949; *J. Biol. Chem.* 184: 661, 1950) indicates that these obligate intracellular parasites possess certain enzymatic mechanisms presumably like those of many biological systems. Studies were undertaken to determine the pathway by which glutamate is oxidized in purified suspensions of *Rickettsia mooseri*. The results show this process follows the steps through the well-known series of dicarboxylic acids. This was elucidated in two ways. Firstly, rickettsial suspensions oxidize the anticipated intermediates, namely, a-ketoglutarate, succinate, fumarate, malate, oxalacetate, and pyruvate. Oxidation of these compounds occurs relatively slowly in comparison with the rate of glutamate oxidation and is demonstrable only in the most active preparations. None of these intermediates is oxidized by normal yolk sac suspensions prepared in the same manner. Secondly, suspensions of *R. mooseri* which are oxidizing glutamate accumulate a-ketoglutarate, succinate, oxalacetate, and pyruvate either spontaneously or in the presence of selective inhibitors. No evidence has yet been obtained for the participation of the tricarboxylic acid cycle except for a very small oxygen consumption of questionable significance with *cis*-aconitate. Since certain antigenic fractions are known to be released from the rickettsiae during preparation, perhaps the failure to demonstrate actively throughout the Krebs cycle may depend on the loss of essential coenzymes washed away during purification.

Complement fixation in diagnosis of human leptospirosis. ROBERT H. YAGER, WILLIAM S. GOCHENOUR, JR., ALBERT R. WARNER,* PSYCHE W. WETMORE* AND HOWARD HALL.* *Army Med. Service Graduate School, Washington, D. C. and Tropical Research Med. Lab., San Juan, Puerto Rico.*

Epidemic leptospirosis in Puerto Rico afforded an opportunity to evaluate complement fixation as a diagnostic procedure. The sonic vibrated leptospiral antigen of Randall (*J. Lab. & Clin. Med.* 34: 1411, 1949), was modified by omission of formalin and then lyophilized. Such antigens retain their original activity after storage at room temperature for at least fourteen months. Undiluted test sera are examined by the method of Kolmer and Boerner (*Approved Laboratory Technic*, 674, 1945) employing half volumes. Anti-

gens prepared from *L. icterohaemorrhagiae*, *L. canicola*, and *L. pomona* each detect antibodies against both heterologous and homologous strains, although evidencing some strain sensitivity in low potency sera. Examination of 50 normal and 22 luetic sera failed to elicit false positive reaction. However, two other luetic sera with cardiolipin titers of 1:128 and 1:64, respectively, gave false positive reactions with leptospiral antigen. Seventy-eight cases of suspected leptospirosis provided a series of 378 sera. Fifty-six cases, comprising 315 sera, were proven leptospirosis by agglutination-lysis test after Schüffner and Mochtar (*Zbl. f. Bakt.* 101: 405, 1927) and/or isolation of Leptospira. Only two cases were not detected by the complement fixation test. Of the 378 sera examined, 6 elicited false positive reactions. The specificity of the complement fixation tests for leptospirosis was not influenced by the presence of luetic antibodies in the sera tested. The present complement fixation test is of value in the clinical diagnosis of leptospirosis.